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NEW POLYNUCLEOTIDES AND
POLYPEPTIDES OF THE IFN α -2 GENE

RELATED APPLICATIONS

- The present invention claims priority to French Patent Application FR 0102843 filed
10 March 1, 2001, titled «Nouveaux Polynucléotides Comportant un Polymorphisme de Type SNP Fonctionnel dans la Séquence Nucléotidique du Gène IFNalpha-2 ainsi que de Nouveaux Polypeptides Codés par ces Polynucléotides et leurs Utilisations Thérapeutiques».

BACKGROUND OF THE INVENTION

15 *Field of the Invention.*

The present invention relates to new polynucleotides derived from the nucleotide sequence of the IFN α -2 gene comprising new SNPs, and new polypeptides derived from the natural IFN α -2 protein comprising mutations caused by these SNPs as well as their therapeutic uses.

20 *Related Art.*

The interferon alpha-2 (IFN α -2) gene is described in the following publications:

- Olopade Ol., Bohlander Sk. "Mapping of the shortest region of overlap of deletions of the short arm of chromosome 9 associated with human neoplasia." Genomics. 1992 Oct; 14(2):437-43;
- Ezekowitz Ra., Mulliken Jb., "Interferon alpha-2a therapy for life-threatening hemangiomas of infancy" N. Engl. J. Med. 1992 May 28; 326(22):1456-63.;
- Dithmar S., Rusciano D., "Neoadjuvant interferon alpha-2b treatment in a murine model for metastatic ocular melanoma: a preliminary study" Arch. Ophthalmol. 2000 Aug; 118(8):1085-9.

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30 The nucleotide sequence of this gene is accessible under accession number J00207, V11834 in the GenBank database.

The IFN α are known for their cellular antiproliferative effects and their involvements in antiviral and antiparasitic responses.

The IFN α are also known to inhibit the expression of several other cytokines at the level

5 of the hematopoietic stem cells, as well as to inhibit the cellular proliferation of certain tumors.

The IFN α are also known to reduce the expression of EGF receptors in renal carcinomas, to inhibit the expression of certain mitochondrial genes, to inhibit the proliferation of fibroblasts, monocytes and B lymphocytes, especially in vitro, and to block the synthesis of antibodies by B lymphocytes.

10 The IFN α are also known to induce the expression of tumor specific antigens on the surface of tumor cells and also to induce genes placed under the control of promoter regions of the ISRE type (Interferon-Stimulated Response Element) by acting on the specific transcription factors of these ISRE.

15 It is known that the IFN α are involved in different disorders and/or human diseases, including but not limited to different cancers, including but not limited to carcinomas, melanomas, lymphomas, leukemias and cancers of the liver, neck, head and kidneys; cardiovascular diseases; metabolic diseases such as those that are not connected with the immune system including obesity; infectious diseases in particular viral infections such as hepatitis B and C and AIDS; pneumonias; ulcerative colitis; diseases of the central nervous system including Alzheimer's disease, schizophrenia and depression; the rejection of tissue or organ grafts; healing of wounds; anemia in dialyzed patients; allergies; asthma; multiple sclerosis; osteoporosis; psoriasis; rheumatoid arthritis; Crohn's disease; autoimmune diseases and disorders; gastrointestinal disorders; and/or disorders connected with chemotherapy treatments.

20 25 The IFN α are particularly used for the treatment of certain leukemias, metastasized renal carcinomas, and tumors that appear following an immunodeficiency, such as Kaposi's sarcoma in the case of AIDS. The IFN α are also effective against other types of tumors and against certain viral infections. The IFN α are also recognized by the FDA (Food and Drug Administration) for the treatment of genital warts or venereal diseases.

30 However, the IFN α , and in particular IFN α -2, have numerous side effects when they are used in pharmaceutical compositions, such as reactions of acute hypersensitivity (urticaria, bronchoconstriction, anaphylactic shock etc.), cardiac arrhythmias, low blood pressure, epileptic seizures, problems with thyroid functions, flu-like syndromes (fevers, sweats, myalgias) etc.

Furthermore, patients treated with IFN α can develop antibodies to these molecules, which neutralize and thus decrease their effectiveness.

5 The present invention is drawn to new polypeptide and polynucleotide analogs to the IFN α -2 gene and its corresponding protein which are capable of having a different functionality from the natural wild-type IFN α -2 protein. In particular, certain of these new polypeptides and new polynucleotides have a cellular antiproliferative activity significantly inhibited by comparison with the natural wild-type IFN α -2.

10 These new polypeptides and polynucleotides can notably be used to treat or prevent the disorders or diseases previously mentioned and avoid all or part of the disadvantages which are tied to them.

BRIEF SUMMARY OF THE INVENTION

15 The present invention is directed to new polynucleotides that differ from the nucleotide sequence of the reference wild-type IFN α -2 gene, in that they comprise one or more SNPs (Single Nucleotide Polymorphism).

20 The nucleotide sequence SEQ ID NO.1 of the human reference wild-type IFN α -2 gene is composed of 1733 nucleotides and comprises a coding sequence of 567 nucleotides, from nucleotide 511 (start codon) to nucleotide 1077 (stop codon).

25 The applicant has identified 9 SNPs in the nucleotide sequence of the reference wild-type IFN α -2 gene.

30 These 9 SNPs are the following: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t.

35 It is understood, in the sense of the present invention, that the numbering corresponds to the positioning of the SNP previously defined and is relative to the nucleotide sequence shown in SEQ ID NO.1. The letters a, t, c and g correspond respectively to the nitrogenous bases adenine, thymine, cytosine and guanine. The first letter corresponds to the wild-type nucleotide, whereas the last letter corresponds to the mutated nucleotide. Thus, for example, the SNP c527a corresponds to a mutation of the nucleotide cytosine (c) at position 527 of the nucleotide sequence SEQ ID NO.1 of the reference wild-type IFN α -2 gene into an adenine (a), and the SNP 96-100del(aattt) corresponds to a mutation in which the 5 nucleotides aattt from positions 96 to 100 of the nucleotide sequence SEQ ID NO.1 of the reference wild-type IFN α -2 gene have been deleted.

40 These SNPs were identified by the applicant using the process described in applicant's

5 patent application entitled "Procedure for the Determination of One or More Functional Polymorphisms," United States Patent Application No. 10/010,749, filed December 6, 2001, fully incorporated here by way of reference.

10 The process described in the 10/010,749 patent application permits the identification of one (or several) preexisting SNP(s) in at least one individual from a random population of individuals.

In the scope of the present invention, two fragments of the nucleotide sequence of the IFN α -2 gene, one of which comprised, the complete coding sequence, were isolated from different individuals in a population of individuals chosen in a random manner.

15 Sequencing of these fragments was then carried out on certain of these samples having a heteroduplex profile (that is a profile different from that of the reference wild-type IFN α -2 gene sequence) after analysis by DHPLC ("Denaturing-High Performance Liquid Chromatography").

The fragment sequenced in this way was then compared to the nucleotide sequence of the fragment of the reference wild-type IFN α -2 gene and the SNPs in conformity with the invention identified.

20 Thus, the SNPs are natural and each of them is present in certain individuals of the world population.

25 The reference wild-type IFN α -2 gene codes for an immature protein of 188 amino acids, corresponding to the amino acid sequence SEQ ID NO. 2, that will be converted to a mature protein of 165 amino acids by cleavage of the signal peptide that includes the first 23 amino acids.

Each of the coding SNPs of the invention, namely c527a and g1023a, causes modifications at the level of the amino acid sequence of the protein encoded by the nucleotide sequence of the IFN α -2 gene.

These modifications in the amino acid sequence are the following:

30 The SNP c527a causes a mutation of the amino acid alanine (A) at position 6 in the immature protein encoded by the IFN α -2 gene, corresponding to the amino acid sequence SEQ ID NO.2, in aspartic acid (D) and is not present in the mature protein since it belongs to the signal sequence. In the description of the present invention the mutation encoded by this SNP will also be called A6D. The SNP c527a affects an amino acid residue located in the signal 35 sequence of the protein. This signal sequence contains all the information necessary for the

5 proper targeting of the mature protein. Thus, the SNP c527a may affect the final localization of the mature protein.

The SNP g1023a causes a mutation of the amino acid methionine (M) at position 171 in the immature protein encoded by the IFN α -2 gene, corresponding to the amino acid sequence SEQ ID NO.2, in isoleucine (I) and at position 148 of the mature protein. In the description of
10 the present invention, the terminology M148I and M171I will be used to refer to the mutation encoded by this SNP according to whether one refers to the mature protein or to the immature protein respectively; the term M148I/M171I will be used where either or both mature and immature proteins are being referred to. The SNP g1023a causes modifications of the spatial conformation of the polypeptides of the present invention as compared to the polypeptide
15 encoded by the nucleotide sequence of the reference wild-type IFN α -2 gene.

The modifications in the spatial conformation can be observed by computational molecular modeling according to methods that are well known to persons skilled in the art, making use of, for example, the modeling tools *de novo* (for example, SEQFOLD/MSI), homology (for example, MODELER/MSI), minimization of the force field (for example, DISCOVER, DELPHI/MSI) and/or molecular dynamics (for example, CFF/MSI). One example of such modeling is given hereinafter in the experimental section.
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Computational molecular modeling permits the observation that the mutation M148I on the mature mutated protein causes a change in the lateral chain near the point of mutation, on helices A and E of IFN α -2. The mutated lateral chain I148 forms a salt bridge with the lateral chain E141, which causes some changes in spatial conformation of the mature mutated protein.
25 Whereas, in the three dimensional conformation of the wild-type IFN α -2, the R144 lateral chain is oriented towards the interior of the molecule, the R144 lateral chain is oriented towards the exterior in the mature mutated protein. Likewise, in the mature mutated protein, the R22 and E141 lateral chains are displaced. Thus, the mutated protein possesses a three-dimensional
30 conformation different from the natural wild-type IFN α -2 protein.

Thus, computational molecular modeling permits the prediction that the presence of the amino acid methionine at position 148 involves a significant modification of the structure and of the function of the reference wild-type IFN α -2 protein.

Other SNPs in conformity with the invention, namely: 96-100del(aattt), t110c, 139-
35 144del(acttta), t338a, t363c, c427t, c1047t, do not involve modification of the protein encoded

- 5 by the nucleotide sequence of the IFN α -2 gene at the level of the amino acid sequence SEQ ID NO. 2.

The SNP c1047t is silent and the SNPs 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t are non-coding.

- 10 Genotyping of the polynucleotides in conformity with the invention can be carried out in such a fashion as to determine the allelic frequency of these polynucleotides in a population. Six examples of genotyping are given, hereinafter, in the experimental section.

15 The determination of the functionality of the polypeptides of the invention can also be carried out by a test of their biological activity. In this regard, it is possible to measure, for example, the antiproliferative effect of polypeptides in conformity with the invention on a Daudi Burkitt's cell line and compare with the natural wild-type IFN α -2 protein. One example of determination of functionality is given hereinafter in the experimental part.

20 The invention is also directed to a method for the use of polynucleotides and polypeptides in conformity with the invention as well as therapeutic molecules obtained and/or identified starting from these polynucleotides and polypeptides, notably for the prevention and the treatment of certain human disorders and/or diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a model of the encoded protein according to the invention comprising the SNP M148I and the natural wild-type protein IFN α -2.

25 Figure 2 represents a close up of the model of the right part of each one of the proteins represented in Figure 1.

The darker ribbon of Figures 1 and 2 represents the structure of the natural wild-type IFN α -2 protein. The lighter ribbon of Figures 1 and 2 represents the structure of the mutated IFN α -2 protein (M148I).

30 Figure 3 represents the results of the test for measuring the antiproliferative effect of a polypeptide of the invention and a polypeptide encoded by the reference wild-type gene of human IFN α -2, on the Daudi Burkitt's cell line. In this figure, the abscissas correspond to the logarithm of the protein concentration in picomoles (pM) and the ordinates correspond to the percentage of cell proliferation. The antiproliferative effect of the wild-type IFN α -2 is

5 represented by triangles and the antiproliferative effect of the M148I mutated IFN α -2 is represented by circles.

Figure 4 represents the survival rate of mice previously infected by VSV virus and treated with M148I mutated IFN α -2, in comparison to those treated with wild-type IFN α -2, or those that have not been treated. In this figure, the abscissas correspond to the relative survival rate of VSV 10 infected mice and the ordinates correspond to the time of survival (days). The black triangles represent the data for VSV infected mice treated with M148I mutated IFN α -2, the black squares represent the data for VSV infected mice treated with wild-type IFN α -2, and the open triangles represent the data for VSV infected mice that have not been treated.

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DETAILED DESCRIPTION OF THE INVENTION

Definitions.

"Nucleotide sequence of the reference wild-type gene" is understood as the nucleotide sequence SEQ ID NO. 1 of the human gene. This sequence is accessible in GenBank under Accession number J00207, V11834 and described in Olopade Ol., Bohlander Sk. "Mapping of the shortest region of overlap of deletions of the short arm of chromosome 9 associated with human neoplasia." Genomics. 1992 Oct;14(2):437-43.

"Natural wild-type IFN α -2 protein" is as understood the mature protein encoded by the nucleotide sequence of the reference wild-type IFN α -2 gene. The natural wild-type immature 25 IFN α -2 protein corresponds to the peptide sequence SEQ ID NO. 2.

"Polynucleotide" is understood as a polyribonucleotide or a polydeoxyribonucleotide that can be a modified or non-modified DNA or an RNA.

The term polynucleotide includes, for example, a single strand or double strand DNA, a DNA comprising a mixture of one or several single strand region(s) and of one or several double 30 strand region(s), a single strand or double strand RNA and an RNA composed of a mixture of one or several single strand region(s) and of one or several double strand region(s). The term polynucleotide can also include an RNA and/or a DNA including one or several triple strand regions. Polynucleotide is equally understood as the DNAs and RNAs containing one or several bases modified in such a fashion as to have a skeleton modified for reasons of stability or for 35 other reasons. A modified base is understood as, for example, the unusual bases such as inosine.

5 "Polypeptide" is understood as a peptide, an oligopeptide, an oligomer or a protein comprising at least two amino acids joined to each other by a normal or modified peptide bond, such as in the cases of the isosteric peptides, for example.

10 A polypeptide according to the invention can comprise amino acids other than the 20 amino acids defined by the genetic code, and can equally be composed of amino acids modified by natural processes, such as post-translational maturation processes or by chemical processes, which are well known to persons skilled in the art. Such modifications are fully detailed in the literature. These modifications can appear anywhere in the polypeptide: in the peptide skeleton, in the amino acid chain, or even at the carboxy- or amino-terminal ends.

15 A polypeptide according to the invention can also be branched following an ubiquitination or be cyclic with or without branching. This type of modification can be the result of natural or synthetic post-translational processes that are well known to persons skilled in the art.

20 Polypeptide modifications may include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent fixation of flavine, covalent fixation of heme, covalent fixation of a nucleotide or of a nucleotide derivative, covalent fixation of a lipid or of a lipidic derivative, the covalent fixation of a phosphatidylinositol, covalent or non-covalent cross-linking, cyclization, disulfide bond formation, demethylation, cysteine formation, pyroglutamate formation, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodization, methylation, myristylation, oxidation, proteolytic processes, phosphorylation, prenylation, racemization, seneyylation, sulfatation, amino acid addition such as arginylation or ubiquitination. Such modifications are fully detailed in the literature; see, e.g., PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, New York, 1993, POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983, Seifter et al. "Analysis for protein modifications and nonprotein cofactors", Meth. Enzymol. (1990) 182: 626-646 and Rattan et al. 25 "Protein Synthesis: Post-translational Modifications and Aging, Ann NY Acad Sci (1992) 663: 48-62.

30 "Isolated polynucleotide" or "isolated polypeptide" are understood as a polynucleotide or a polypeptide such as previously defined which is isolated from the human body or otherwise 35 produced by a technical process.

5 "Identity" is understood as the measurement of nucleotide or polypeptide sequence identity. Identity is a term well known to persons skilled in the art and well described in the literature. See, e.g., COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., Ed., Oxford University Press, New York, 1998; BIOCOMPUTING INFORMATICS AND GENOME PROJECT, Smith, D.W., Ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF
10 SEQUENCE DATA, PART I, Griffin, A.M. and Griffin H.G., Ed, Humana Press, New Jersey, 1994; et SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987. The methods commonly employed to determine the identity and the similarity between two sequences are equally well described in the literature. See, e.g., GUIDE TO HUGE COMPUTER, Martin J. Bishop, Ed, Academic Press, San Diego, 1994, and Carillo H. and
15 Lipton D., Siam J Applied Math (1988) 48: 1073.

A polynucleotide having, for example, an identity of at least 95 % with the nucleotide sequence SEQ ID NO. 1 is a polynucleotide which contains at most 5 points of mutation over 100 nucleotides, compared to said sequence. These points of mutation can be one (or several) substitution(s), addition(s) and/or deletion(s) of one (or several) nucleotide(s).

In the same way, a polypeptide having, for example, an identity of at least 95 % with the amino acid sequence SEQ ID NO. 2 is a polypeptide that contains at most 5 points of mutation over 100 amino acids, compared to said sequence. These points of mutation can be one (or several) substitution(s), addition(s) and/or deletion(s) of one (or several) amino acid(s).

The polynucleotides and the polypeptides according to the invention which are not totally identical with, respectively, the nucleotide sequence SEQ ID N°1 or the amino acid sequence SEQ ID N°2, containing at least one of the SNPs of the invention, are considered as variants of these sequences.

In general, polynucleotides according to the invention possess the same or practically the same biological activity as the nucleotide sequence SEQ ID NO. 1 comprising at least one of the SNPs of the invention.

In similar fashion, polypeptides according to the invention generally possess the same or practically the same biological activity as the amino acid sequence SEQ ID NO. 2 comprising at least one of the coding SNPs of the invention.

A variant according to the invention can be obtained, for example, by site-directed mutagenesis or by direct synthesis.

5 "SNP" is understood as any natural variation of a base in a nucleotide sequence. A SNP in a nucleotide sequence can be coding, silent or non-coding.

10 A coding SNP is a polymorphism in the coding sequence of a nucleotide sequence that involves a modification of one or more amino acids in the sequence of amino acids encoded by this nucleotide sequence. In this case, the term SNP applies equally, by extension, to a mutation in an amino acid sequence.

A silent SNP is a polymorphism in the coding sequence of a nucleotide sequence that does not involve a modification of any amino acid in the amino acid sequence encoded by this nucleotide sequence.

15 A non-coding SNP is a polymorphism in the non-coding sequence of a nucleotide sequence. This polymorphism can notably be found in an intron, a splicing zone, a transcription promoter or an enhancer site sequence.

"Functional SNP" is understood as a SNP, such as previously defined, which is included in a nucleotide sequence or an amino acid sequence, and has some functionality.

20 "Functionality" is understood as the biological activity of a polypeptide or of a polynucleotide.

The functionality of a polypeptide or of a polynucleotide according to the invention can consist in a conservation, an augmentation, a reduction or a suppression of the biological activity of the polypeptide encoded by the nucleotide sequence of the reference wild-type gene or of this latter nucleotide sequence.

25 The functionality of a polypeptide or of a polynucleotide according to the invention can also consist of a change in the nature of the biological activity of the polypeptide encoded by the nucleotide sequence of the reference wild-type gene or of this latter nucleotide sequence.

The biological activity can, notably, be linked to the affinity or to the absence of affinity of a polypeptide according to the invention with a receptor.

30

Polynucleotides.

The present invention is directed to isolated polynucleotides comprising:

35 a) a nucleotide sequence having at least 80% identity, preferably at least 90% identity, more preferably at least 95% identity and still more preferably at least 99% identity with the sequence SEQ ID NO. 1 or its coding sequence (nucleotide 511 to nucleotide 1077), it being

5 understood that this nucleotide sequence comprises at least one of the following coding SNPs: c527a or g1023a.

b) a nucleotide sequence complementary to a nucleotide sequence under a).

The present invention relates equally to an isolated polynucleotide comprising:

a) the nucleotide sequence SEQ ID NO. 1 or its coding sequence, it being understood that

10 each of these sequences comprises at least one of the following coding SNPs: c527a or g1023a.

b) a nucleotide sequence complementary to a nucleotide sequence under a).

Preferably, the polynucleotide of the invention consists of the sequence SEQ ID NO. 1 or its coding sequence, it being understood that each of these sequences comprises at least one of the following coding SNPs: c527a, g1023a.

15 According to the invention, the polynucleotide previously defined comprises a single coding SNP selected from the group consisting of: c527a and g1023a.

A polynucleotide such as previously defined can also include at least one of the following non-coding and silent SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c1047t.

20 The present invention is also directed to isolated polynucleotides comprising or consisting of:

a) nucleotide sequence SEQ ID NO. 1 or its coding sequence, such that each of these sequences comprises at least one of the following non-coding or silent SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t or c1047t; and/or

25 b) a nucleotide sequence complementary to a nucleotide sequence under a).

It is understood that only the silent SNP c1047t is located in the coding sequence of the nucleotide sequence SEQ ID NO. 1.

The present invention also concerns an isolated polynucleotide consisting of a part of:

a) nucleotide sequence SEQ ID NO. 1 or its coding sequence, such that each one of these

30 sequences contains at least one of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t; or

b) a nucleotide sequence complementary to a nucleotide sequence under a);

said isolated polynucleotide being composed of at least 10 nucleotides.

Preferably, the isolated polynucleotide as defined above is composed of 10 to 40

35 nucleotides.

5 The present invention is also directed to isolated polynucleotides coding for a polypeptide comprising:

a) the amino acid sequence SEQ ID NO. 2, or

b) the amino acid sequence comprising the amino acids included between positions 24 and 188 of the sequence of amino acids SEQ ID NO. 2,

10 it being understood that each of the amino acid sequences under a) and b) comprises at least one of the following coding SNPs: A6D, M171I.

It is understood, in the sense of the present invention, that the numbering corresponding to the positioning of the A6D and M171I SNPs is relative to the numbering of the amino acid sequence SEQ ID NO.2.

15 In a preferred embodiment of the invention, the previously defined polypeptide comprises a single coding SNP such as defined above.

Preferably, a polynucleotide according to the invention is composed of a DNA or RNA molecule.

20 A polynucleotide according to the invention can be obtained by standard DNA or RNA synthetic methods.

A polynucleotide according to the invention can equally be obtained by site-directed mutagenesis starting from the nucleotide sequence of the IFN α -2 gene by modifying the wild-type nucleotide to the mutated nucleotide for each SNP on the nucleotide sequence SEQ ID NO. 1.

25 For example, a polynucleotide according to the invention, comprising SNP g1023a can be obtained by site-directed mutagenesis starting from the nucleotide sequence of the IFN α -2 gene by changing the guanosine at position 1023 in the nucleotide sequence SEQ ID NO.1 to adenosine.

The processes of site-directed mutagenesis that can be implemented in this way are well known to persons skilled in the art, see, e.g., the publication of TA Kunkel in 1985 in "Proc. Natl. Acad. Sci. USA" 82:488.

An isolated polynucleotide can equally include, for example, nucleotide sequences coding for pre-, pro- or pre-pro-protein amino acid sequences or marker amino acid sequences, such as hexa-histidine peptide.

5 A polynucleotide of the invention can equally be associated with nucleotide sequences coding for other proteins or protein fragments in order to obtain fusion proteins or other purification products.

10 A polynucleotide according to the invention can also include nucleotide sequences such as the 5' and/or 3' non-coding sequences, such as, for example, transcribed or non-transcribed sequences, translated or non-translated sequences, splicing signal sequences, polyadenylated sequences, ribosome binding sequences or even sequences which stabilize mRNA.

A nucleotide sequence complementary to the nucleotide or polynucleotide sequence is defined as one that can hybridize with this nucleotide sequence, under stringent hybridization conditions.

15 "Stringent hybridization conditions" is generally but not necessarily understood as the chemical conditions that permit hybridization only when the nucleotide sequences have an identity of at least 80%, preferably greater than or equal to 90%, still more preferably greater than or equal to 95% and most preferably greater than or equal to 97%.

The stringent conditions can be obtained according to methods well known to persons skilled in the art,, for example, by incubation of the polynucleotides, at 42° C, in a solution comprising 50% formamide, 5xSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH = 7.6), 5x Denhardt Solution, 10% dextran sulfate and 20 µg denatured salmon sperm DNA, followed by washing the filters at 0.1x SSC, at 65° C.

20 Within the scope of the invention, when the stringent hybridization conditions only permit hybridization of the nucleotide sequences having an identity equal to 100%, the nucleotide sequence is considered to be strictly complementary to the nucleotide sequence such as described under a).

25 It is understood within the meaning of the present invention that the nucleotide sequence complementary to a nucleotide sequence comprises at least one anti-sense SNP according to the invention.

30 Thus, for example, if the nucleotide sequence comprises the SNP g1023a, its complementary nucleotide sequence comprises the t nucleotide at the equivalent of position 1023.

5 *Identification, hybridization and/or amplification of a polynucleotide comprising a SNP.*

The present invention also has for its object a method for the use of all or part of:

a) a polynucleotide having 80 to 100% identity with the nucleotide sequence SEQ ID

NO. 1, and/or

b) a polynucleotide according to the invention comprising at least one SNP;

10 in order to identify, hybridize and/or amplify all or part of a polynucleotide having 80 to 100% identity with the nucleotide sequence SEQ ID NO. 1 or if necessary its coding sequence (of the nucleotide 511 to the nucleotide 1077), it being understood that each one of these sequences contains at least one of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t.

15

Genotyping and determination of the frequency of a SNP.

The present invention equally has for its object a method for the use of all or part of:

a) a polynucleotide having 80 to 100% identity (preferably at least 90% identity, more preferably 95% identity and particularly 100% identity) with the nucleotide sequence SEQ ID NO.1, and/or

b) a polynucleotide according to the invention comprising at least one SNP,

for the genotyping of all or part of a polynucleotide having 80 to 100% identity (preferably at least 90% identity, more preferably 95% identity and particularly 100% identity) with the nucleotide sequence SEQ ID NO. 1 or if necessary its coding sequence (of the nucleotide 511 to the nucleotide 1077), it being understood that each one of these sequences comprises at least one of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t.

20 According to the invention, the genotyping may be carried out on an individual or a population of individuals, most preferably in a population of individuals. A genotype consists of
30 the alleles present at one or more specific loci. Within the meaning of the invention, genotyping is defined as a process for the determination of the genotype of an individual or of a population of individuals.

“Population of individuals” (also referred to as “population of subjects”) is understood as a group of determined individuals selected in random or non-random fashion. These individuals
35 (or subjects) can be humans, animals, microorganisms or plants. Usually, the group of

5 individuals comprises at least 10 persons, preferably from 100 to 300 persons.

The individuals can be selected according to their ethnicity or according to their phenotype, including those who are affected by the following disorders and/or diseases: cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, 10 and disorders connected with chemotherapy treatments.

Said cancers and tumors include carcinomas comprising metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and 15 tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.

Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.

Said metabolic diseases may include such non-immune associated diseases as obesity.

Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's disease, schizophrenia and depression.

25 Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.

Multiple technologies exist which can be implemented in order to genotype SNPs (see, for example, Kwok Pharmacogenomics, 2000, vol 1, pp 95-100. "High-throughput genotyping assay approaches"). These technologies are based on one of the four following principles: allele specific 30 oligonucleotide hybridization, oligonucleotide elongation by dideoxynucleotides optionally in the presence of deoxynucleotides, ligation of allele specific oligonucleotides, or cleavage of allele specific oligonucleotides. Each one of these technologies can be coupled to a detection system such as measurement of direct or polarized fluorescence, or mass spectrometry.

35 Genotyping can notably be carried out by minisequencing with hot ddNTPs (2 different ddNTPs labeled by different fluorophores) and cold ddNTPs (2 different non labeled ddNTPs),

5 in connection with a polarized fluorescence scanner. The minisequencing protocol involving
detection of polarized fluorescence (FP-TDI Technology or Fluorescence Polarization Template-
direct Dye-Terminator Incorporation) is well known to persons skilled in the art. FP-TDI can be
carried out on a product obtained by amplification by polymerase chain reaction (PCR) of the
DNA of each individual. This PCR product is selected to cover the polynucleotide genic region
10 containing the studied SNP. After the last step in the PCR thermocycler, the plate is then placed
on a polarized fluorescence scanner for reading of the labeled bases using fluorophore-specific
excitation and emission filters. The intensity values of the labeled bases are reported on a graph.

For the PCR amplification, in the case of a SNP of the invention, the sense and antisense
primers, respectively, can easily be selected by persons skilled in the art according to the position
15 of the SNPs of the invention in order to amplify a region of interest containing the SNP or SNPs.

For example, a first set of sense and antisense nucleotide sequences for the PCR
amplification can be:

SEQ ID NO. 3: Sense primer: GCCTCTTATGTACCCACAAA

SEQ ID NO. 4: Antisense primer: CACCAGTAAAGCAAAGGTCA

These nucleotide sequences permit amplification of a fragment having a length of 535
nucleotides, comprised of nucleotide 3 to nucleotide 537 in the nucleotide sequence SEQ ID
NO. 1.

For example, a second set of sense and antisense nucleotide sequences for the PCR
amplification can be:

SEQ ID NO. 5: Sense primer: CACCCATTCAACCAGTCTA

SEQ ID NO. 6: Antisense primer: AGCTGGCATACGAATCAAT

These nucleotide sequences permit amplification of a fragment having a length of 655
nucleotides, comprised of nucleotide 470 to nucleotide 1124 in the nucleotide sequence SEQ ID
NO. 1.

30 A statistical analysis of the frequency of each allele (allelic frequency) encoded by the
gene comprising the SNP in the population of individuals is then achieved, which permits
determination of the importance of their impact and their distribution in the different sub-groups,
including the diverse ethnic groups that constitute this population of individuals.

The genotyping data are analyzed in order to estimate the distribution frequency of the
35 different alleles observed in the studied populations. The calculations of the allelic frequencies

5 can be carried out with the help of software such as SAS-suite® (SAS) or SPLUS® (MathSoft). The comparison of the allelic distributions of a SNP of the invention across different ethnic groups of the population of individuals can be carried out by means of software such as ARLEQUIN® and SAS-suite®.

10 *SNPs of the invention as genetic markers.*

Whereas SNPs modifying functional sequences of genes (e.g. promoter, splicing sites, coding region) are likely to be directly related to disease susceptibility or resistance, all SNPs (functional or not) may provide valuable markers for the identification of one or several genes involved in these disease states and, consequently, may be indirectly related to these disease states (See Cargill et al. (1999). Nature Genetics 22:231-238; Riley et al. (2000). Pharmacogenomics 1:39-47; Roberts L. (2000). Science 287: 1898-1899).

15 Thus, the present invention also concerns a databank comprising one or more of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t, in a polynucleotide of the IFN α -2 gene.

20 It is well understood that said SNPs are numbered in accordance with the nucleotide sequence SEQ ID NO. 1.

This databank may be analyzed for determining statistically relevant associations between:

25 (i) one or more of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, and/or c1047t in a polynucleotide of the IFN α -2 gene, and
(ii) a disease or a resistance to a disease.

The present invention also concerns the use of one or mroe of the following SNPs: 96-100del(aattt), t110c, 139-144del(acttta), t338a, t363c, c427t, c527a, g1023a, c1047t, in a polynucleotide of the IFN α -2 gene, for developing diagnostic/prognostic kits for a disease or a
30 resistance to a disease.

A SNP of the invention such as defined above may be directly or indirectly associated to a disease or a resistance to a disease.

Preferably, these diseases may be those which are defined above.

5 *Expression vector and host cell.*

The present invention also has for its object a recombinant vector comprising one or more polynucleotide according to the invention.

Numerous expression systems can be used, including, without limitation chromosomes, episomes, and/or derived viruses. More particularly, the recombinant vectors used can be derived from bacterial plasmids, transposons, yeast episome, insertion elements, yeast chromosome elements, viruses such as baculovirus, papilloma viruses such as SV40, vaccinia viruses, adenoviruses, fox pox viruses, pseudorabies viruses, retroviruses.

These recombinant vectors can equally be cosmid or phagemid derivatives. The nucleotide sequence can be inserted in the recombinant expression vector by methods well known to persons skilled in the art such as, for example, those that are described in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

The recombinant vector can include nucleotide sequences that control the regulation of the polynucleotide expression as well as nucleotide sequences permitting the expression and the transcription of a polynucleotide of the invention and the translation of a polypeptide of the invention, these sequences being selected according to the host cells that are used.

Thus, for example, an appropriate secretion signal can be integrated in the recombinant vector so that the polypeptide, encoded by the polynucleotide of the invention, will be directed towards the lumen of the endoplasmic reticulum, towards the periplasmic space, to the membrane or towards the extracellular environment.

The present invention also has for its object a host cell comprising a recombinant vector according to the invention.

The introduction of the recombinant vector in a host cell can be carried out according to methods that are well known to persons skilled in the art such as those described in BASIC METHODS IN MOLECULAR BIOLOGY, Davis et al., 1986 and MOLECULAR CLONING: A LABORATORY MANUAL, (supra), such as transfection by calcium phosphate, transfection by DEAE dextran, transfection, microinjection, transfection by cationic lipids, electroporation, transduction or infection.

The host cell can be, for example, bacterial cells such as cells of streptococci, staphylococci, *E. coli* or *Bacillus subtilis*, cells of fungi such as yeast cells and cells of

- 5 *Aspergillus*, *Streptomyces*, insect cells such as cells of *Drosophila* S2 and of *Spodoptera* Sf9, animal cells, such as CHO, COS, HeLa, C127, BHK, HEK 293 cells and human cells of the subject to treat or even plant cells.

The host cells can be used, for example, to express a polypeptide of the invention or as an active product in pharmaceutical compositions, as will be seen hereinafter.

10

Polypeptides.

The present invention also has for its object an isolated polypeptide comprising an amino acid sequence having at least 80% identity, preferably at least 90% identity, more preferably at least 95% identity and still more preferably at least 99% identity with:

15

a) the amino acid sequence SEQ ID NO. 2 or with

b) the amino acid sequence comprising the amino acids included between positions 24 and 188 of the amino acid sequence SEQ ID NO. 2,

it being understood that each of the amino acid sequences under a) and b) contains at least one of the following coding SNPs: A6D, M171I.

The polypeptide of the invention can equally comprise:

a) the amino acid sequence SEQ ID NO. 2, or

b) the amino acid sequence containing the amino acids included between positions 24 and 188 of the amino acid sequence SEQ ID NO. 2,

it being understood that each of the amino acid sequences under a) and b) contains at least one of the following coding SNPs: A6D, M171I.

The polypeptide of the invention can more particularly consist of:

a) the amino acid sequence SEQ ID NO. 2, or

b) the amino acid sequence containing the amino acids included between positions 24 and 188 of the amino acid sequence SEQ ID NO. 2,

30

it being understood that each one of the amino acid sequences under a) and b) contains at least one of the following coding SNPs: A6D, M171I.

Preferably, a polypeptide according to the invention contains a single coding SNP selected from the group consisting of A6D and M171I.

5 The present invention equally has for its object a process for the preparation of the above-described polypeptide, in which a previously defined host cell is cultivated in a culture medium and said polypeptide is isolated from the culture medium.

10 The polypeptide can be purified starting from the host cells' culture medium, according to methods well known to persons skilled in the art such as precipitation with chaotropic agents such as salts, in particular ammonium sulfate, ethanol, acetone or trichloroacetic acid, acid extraction; ion exchange chromatography; phosphocellulose chromatography; hydrophobic interaction chromatography; affinity chromatography; hydroxyapatite chromatography or exclusion chromatographies.

15 “Culture medium” is understood as the medium in which the polypeptide of the invention is isolated or purified. This medium can be composed of the extracellular medium and/or the cellular lysate. Techniques well known to persons skilled in the art also permit the latter to produce an active conformation to the polypeptide, if the conformation of said polypeptide was altered during the isolation or the purification.

20 *Antibodies.*

25 The present invention also concerns a method for obtaining an immunospecific antibody.

30 “Antibody” is understood as the monoclonal, polyclonal, chimeric, simple chain, and/or humanized antibodies as well as the Fab fragments, including Fab or immunoglobulin expression library products.

35 An immunospecific antibody can be obtained by immunization of an animal with a polypeptide according to the invention.

40 The invention also relates to an immunospecific antibody for a polypeptide according to the invention, such as defined previously.

45 A polypeptide according to the invention, one of its fragments, an analog, one of its variants or a cell expressing this polypeptide can also be used to produce immunospecific antibodies.

50 The term “immunospecific” means that the antibody possesses a better affinity for the polypeptide of the invention than for other polypeptides known in the prior art.

55 The immunospecific antibodies can be obtained by administration of a polypeptide of the invention, of one of its fragments, of an analog or of an epitopic fragment or of a cell expressing

- 5 this polynucleotide in a mammal, preferably non human, according to methods well known to persons skilled in the art.

For the preparation of monoclonal antibodies, typical methods for antibody production can be used, starting from cell lines, such as the hybridoma technique (Kohler et al., Nature 1975) 256: 495-497), the trioma technique, the human B cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4: 72), and the EBV hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, 1985).

The techniques of single chain antibody production such as described, for example, in US Patent No. 4,946, 778 can also be used.

Transgenic animals, such as mice, for example, can also be used to produce humanized antibodies.

Agents interacting with the polypeptide of the invention.

The present invention equally has for its object a method for the identification of an agent which activates or inhibits a polypeptide according to the invention, comprising:

a) the preparation of a recombinant vector comprising a polynucleotide according to the invention containing at least one coding SNP,

b) the preparation of host cells comprising a recombinant vector according to a),

c) the contacting of host cells according to b) with an agent to be tested, and

d) the determination of the activating or inhibiting effect generated by the agent to test.

A polypeptide according to the invention can also be employed for screening compounds that interact with it.

These compounds can be activating (agonists) or inhibiting (antagonists) agents of intrinsic activity of a polypeptide according to the invention. These compounds can also be ligands or substrates of a polypeptide of the invention. See Coligan et al., Current Protocols in Immunology 1 (2), Chapter 5 (1991).

In general, in order to implement such a process, it is first desirable to produce appropriate host cells that express a polypeptide according to the invention. Such cells can be, for example, cells of mammals, yeasts, insects such as *Drosophila* or bacteria such as *E. coli*.

These cells or membrane extracts of these cells are then put in the presence of compounds to be tested.

5 The binding capacity of the compounds to be tested with the polypeptide of the invention can then be observed, as well as the inhibition or the activation of the functional response.

Step d) of the above process can be implemented by using an agent to be tested that is directly or indirectly labeled. It can also include a competition test, by using a labeled or non-labeled agent and a labeled competitor agent.

10 It can also be determined if an agent to be tested generates an activation or inhibition signal on cells expressing the polypeptide of the invention by using detection means appropriately chosen according to the signal to be detected.

Such activating or inhibiting agents can be polynucleotides, and in certain cases oligonucleotides or polypeptides, such as proteins or antibodies, for example.

15 The present invention is also directed to a method for the identification of an agent activated or inhibited by a polypeptide according to the invention, comprising:

- a) the preparation of a recombinant vector comprising a polynucleotide according to the invention containing at least one coding SNP,
- b) the preparation of host cells comprising a recombinant vector according to a),
- c) placing host cells according to b) in the presence of an agent to be tested, and
- d) the determination of the activating or inhibiting effect generated by the polypeptide on the agent to be tested.

An agent activated or inhibited by the polypeptide of the invention is an agent that responds, respectively, by an activation or an inhibition in the presence of this polypeptide. The agents, activated or inhibited directly or indirectly by the polypeptide of the invention, can consist of polypeptides such as, for example, membrane-bound or nuclear receptors, kinases including tyrosine kinases, transcription factors or polynucleotides.

Detection of diseases.

30 The present invention also has for an object a method for analyzing the biological characteristics of a polynucleotide according to the invention and/or of a polypeptide according to the invention in a subject, comprising at least one of the following:

- a) Determining the presence or the absence of a polynucleotide according to the invention in the genome of a subject;
- b) Determining the level of expression of a polynucleotide according to the invention in a

5 subject;

c) Determining the presence or the absence of a polypeptide according to the invention in a subject;

d) Determining the concentration of a polypeptide according to the invention in a subject; and/or

10 e) Determining the functionality of a polypeptide according to the invention in a subject.

These biological characteristics may be analyzed in a subject or in a sample from a subject.

These biological characteristics may permit a genetic diagnosis to determine whether a subject is affected or at risk of being affected, or, on the contrary, presents a partial resistance to the development of a disease, an indisposition or a disorder linked to the presence of a polynucleotide according to the invention and/or a polypeptide according to the invention.

These diseases can be disorders and/or human diseases, such as cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, and disorders connected with chemotherapy treatments.

Said cancers and tumors include carcinomas comprising metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.

Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.

Said metabolic diseases may include such non-immune associated diseases as obesity.

Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's disease, schizophrenia and depression.

Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.

5 This process also permits genetic diagnosis of a disease or of a resistance to a disease linked to the presence, in a subject, of the mutant allele encoded by a SNP according to the invention.

Preferably, in step a), the presence or absence of a polynucleotide, containing at least one coding SNP such as previously defined, will be detected.

10 The detection of the polynucleotide may be carried out starting from biological samples from the subject to be studied, such as cells, blood, urine, saliva, or starting from a biopsy or an autopsy of the subject to be studied. Genomic DNA may be used for detection directly or after a PCR amplification, for example. RNA or cDNA can equally be used in a similar fashion.

15 It is then possible to compare the nucleotide sequence of a polynucleotide according to the invention with the nucleotide sequence detected in the genome of the subject. The comparison of the nucleotide sequences can be carried out by sequencing, by DNA hybridization methods, by mobility difference of the DNA fragments on an electrophoresis gel with or without denaturing agents or by melting temperature difference. See Myers et al., Science (1985) 230: 1242. Such modifications in the structure of the nucleotide sequence at a precise point can equally be revealed by nuclease protection tests, such as RNase and the S1 nuclease or also by chemical cleaving agents. See Cotton et al., Proc. Nat. Acad. Sci. USA (1985) 85: 4397-4401. Oligonucleotide probes comprising a polynucleotide fragment of the invention can equally be used to conduct the screening.

20 Many methods well known to persons skilled in the art can be used to determine the expression of a polynucleotide of the invention and to identify the genetic variability of this polynucleotide (See Chee et al., Science (1996), Vol 274, pp 610-613).

25 In step b), the level of expression of the polynucleotide may be measured by quantifying the level of RNA encoded by this polynucleotide (and coding for a polypeptide) according to methods well known to persons skilled in the art such as, for example, by PCR, RT-PCR, RNase protection, Northern blot, and other hybridization methods.

30 In step c) and d) the presence or the absence as well as the concentration of a polypeptide according to the invention in a subject or a sample form a subject may be carried out by well known methods such as, for example, by radioimmunoassay, competitive binding tests, Western blot and ELISA tests.

5 Consecutively to step d), the determined concentration of the polypeptide according to the invention can be compared with the natural wild-type protein concentration usually found in a subject.

10 Persons skilled in the art can identify the threshold above or below which appears the sensitivity or, to the contrary, the resistance to the disease, the indisposition or the disorder evoked above, with the help of prior art publications or by conventional tests or assays, such as those that are previously mentioned.

15 In step e), the determination of the functionality of a polypeptide according to the invention may be carried out by methods well known to persons skilled in the art such as, for example, by in vitro tests such as above mentioned or by an use of host cells expressing said polypeptide.

Medications and treatments of diseases.

20 The polypeptides of the invention possess very interesting pharmacological properties. In particular, they can bind to the human IFN α -2 receptor. These properties are in accordance with the use of the polypeptides of the invention for therapeutic treatment of human body, i.e. as a therapeutic composition.

25 Thus, the present invention also has for its object a therapeutic compound comprising, as an active agent, a polypeptide according to the invention.

30 The invention also relates to a method for the use of a polypeptide according to the invention, for the manufacture of a therapeutic composition intended for the prevention or the treatment of different human disorders and/or diseases. These diseases can be disorders and/or human diseases, such as cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, and disorders connected with chemotherapy treatments.

35 Said cancers and tumors include carcinomas comprising metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

- 5 Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.
- Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.
- 10 Said metabolic diseases may include such non-immune associated diseases as obesity.
- Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's disease, schizophrenia and depression.
- Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.
- 15 Preferably, a polypeptide according to the invention can also be used for the manufacture of a therapeutic composition intended for the prevention or the treatment of different human disorders and/or diseases, such as certain leukemias, metastasized renal carcinomas as well as tumors that appear following an immunodeficiency, such as Kaposi's sarcoma in the case of AIDS, other types of tumors, certain viral infections, and/or genital warts or other venereal diseases.
- Certain of the compounds comprising the polypeptide according to the invention, as well as the compounds obtained or identified by or from this polypeptide, can likewise be used for the therapeutic treatment of the human body, i.e. as a medicament.
- 20 This is why the present invention also has for an object a therapeutic composition comprising, as an active agent, a polynucleotide according to the invention comprising at least one previously defined coding SNP, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody.
- 25 The invention also relates to a method for the use of a polynucleotide according to the invention containing at least one previously defined coding SNP, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, for the manufacture of a therapeutic composition intended for the prevention or the treatment of different human disorders and/or diseases, such as cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, and disorders connected with chemotherapy treatments.
- 30 Said cancers and tumors, may include carcinomas comprising metastasizing renal
- 35

5 carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

10 Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.

Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.

15 Said metabolic diseases may include such non-immune associated diseases as obesity.

Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's disease, schizophrenia and depression.

Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.

20 Preferably, the invention concerns a method for the use of a polynucleotide according to the invention containing at least one previously defined SNP, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, for the manufacture of a therapeutic composition intended for the prevention or the treatment of different human disorders and/or diseases, such as certain leukemias, metastasized renal carcinomas as well as tumors that appear following an immunodeficiency, such as Kaposi's sarcoma in the case of AIDS, other types of tumors, certain viral infections, and genital warts or other venereal diseases.

25 The dosage of a polypeptide and of the other compounds of the invention, useful as active agent, depends on the choice of the compound, the therapeutic indication, the mode of administration, the nature of the formulation, the nature of the subject and the judgment of the doctor.

30 When it is used as active agent, a polypeptide according to the invention is generally administered at doses ranging between 1 and 100 µg/kg of the subject.

The invention also has as an object a pharmaceutical composition that contains, as active agent, at least one above-mentioned compound such as a polypeptide according to the invention, 35 a polynucleotide according to the invention containing at least one previously defined SNP, a

- 5 previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, as well as a pharmaceutically acceptable excipient.

In these pharmaceutical compositions, the active agent is advantageously present at physiologically effective doses.

- These pharmaceutical compositions can be, for example, solids or liquids and be present
10 in pharmaceutical forms currently used in human medicine such as, for example, simple or coated tablets, gelcaps, granules, caramels, suppositories and preferably injectable preparations and powders for injectables. These pharmaceutical forms can be prepared according to usual methods.

- The active agent(s) can be incorporated into excipients usually employed in
15 pharmaceutical compositions such as talc, Arabic gum, lactose, starch, dextrose, glycerol, ethanol, magnesium stearate, cocoa butter, aqueous or non-aqueous vehicles, fatty substances of animal or vegetable origin, paraffinic derivatives, glycols, various wetting agents, dispersants or emulsifiers, preservatives.

The active agent(s) according to the invention can be employed alone or in combination with other compounds such as therapeutic compounds including without limitation, other interferons- α or other cytokines such as interleukine, for example.

The different formulations of the pharmaceutical compositions are adapted according to the mode of administration.

The pharmaceutical compositions can be administered by different routes of administration known to persons skilled in the art.

- 25 The invention also relates to a diagnostic composition that contains, as active agent, at least one above-mentioned compound such as a polypeptide according to the invention, all or part of a polynucleotide according to the invention, a previously defined recombinant vector, a previously defined host cell, and/or a previously defined antibody, as well as a suitable pharmaceutical acceptable excipient.

30 This diagnostic composition may contain, for example, an appropriate excipient like those generally used in the diagnostic composition such as buffers and preservatives.

The present invention is also directed to a method of the use of:

- 35 a) a therapeutically effective quantity of a polypeptide according to the invention, and/or
b) a polynucleotide according to the invention, and/or

5 c) a host cell from the subject to be treated, previously defined,
to prepare a therapeutic composition intended to increase the expression or the activity, in a
subject, of a polypeptide according to the invention.

Thus, to treat a subject who needs an increase in the expression or in the activity of a polypeptide of the invention, several methods are possible.

10 It is possible to administer to the subject a therapeutically effective quantity of a polypeptide of the invention with a pharmaceutically acceptable excipient.

15 It is likewise possible to increase the endogenous production of a polypeptide of the invention by administration to the subject of a polynucleotide according to the invention. For example, this polynucleotide can be inserted in a retroviral expression vector. Such a vector can be isolated starting from cells having been infected by a retroviral plasmid vector containing RNA encoding for the polypeptide of the invention, in such a fashion that the transduced cells produce infectious viral particles containing the gene of interest. See Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, Chapter 20, in Human Molecular Genetics, Strachan and Read, BIOS Scientifics Publishers Ltd (1996).

20 In accordance with the invention, a polynucleotide containing at least one coding SNP such as previously defined will preferably be used.

25 It is equally possible to administer to the subject host cells belonging to him, these host cells having been preliminarily taken and modified so as to express the polypeptide of the invention, as previously described.

The present invention equally relates to the use:

- a) of a therapeutically effective quantity of a previously defined immunospecific antibody, and/or
- b) of a polynucleotide permitting inhibition of the expression of a polynucleotide according to the invention,

30 in order to prepare a medicament intended to reduce the expression or the activity, in a subject, of a polypeptide according to the invention.

Thus, it is possible to administer to the subject a therapeutically effective quantity of an inhibiting agent and/or of an antibody such as previously defined, possibly in combination, with a pharmaceutically acceptable excipient.

5 It is equally possible to reduce the endogenous production of a polypeptide of the invention by administration to the subject of a complementary polynucleotide according to the invention permitting inhibition of the expression of a polynucleotide of the invention.

Preferably, a complementary polynucleotide containing at least one coding SNP such as previously defined can be used.

10 The present invention concerns also the use of a IFN α -2 protein for the preparation of a therapeutic composition for the prevention or the treatment of a patient having a disorder or a disease caused by an IFN α -2 variant linked to the presence in the genome of said patient of a nucleotide sequence having at least 95% identity (preferably, 97% identity, more preferably 99% identity and particularly 100% identity) with the nucleotide sequence SEQ ID NO. 1, provided
15 that said nucleotide sequence comprises one of the following SNPs: 96-100del(aattt), t110c, 139-144del(actta), t338a, t363c, c427t, c527a, g1023a, or c1047t.

Preferably, said therapeutic composition is used for the prevention or the treatment of one of the diseases selected from the group consisting of disorders and/or human diseases, including cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, and disorders connected with chemotherapy treatments.

Said cancers and tumors may include carcinomas comprising metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.

30 Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.

Said metabolic diseases may include such non-immune associated diseases as obesity.

35 Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's disease, schizophrenia and depression.

5 Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.

Mimetic compounds of a IFN α -2 polypeptide comprising the SNP M171I.

10 The present invention also concerns a new compound having a biological activity substantially similar or lower in comparison to that of the polypeptide of:

- a) amino acid sequence SEQ ID NO.2, or
 - b) amino acid sequence comprising the amino acids between positions 24 and 188 of the amino acid sequence SEQ ID NO.2;
- provided that said amino acid sequences under a) and b) comprise the M171I SNP.

15 Said biological activity may be evaluated, for example, by measuring cellular antiproliferative activity on Daudi Burkitt's cell line or signal transduction assays on MCF7 cells using luciferase reporter gene as described below in the experimental section.

20 As mentioned in the experimental section, the M171I mutated IFN α -2 possesses a cellular antiproliferative activity on Daudi Burkitt's cell line which is lower than that of the natural wild-type IFN α -2.

As mentioned in the experimental section, the signal transduction activation in the breast carcinoma cell line MCF-7 measured in presence of the M171I mutated IFN α -2 is lower than that measured with the natural wild-type IFN α -2.

25 A new compound of the invention, such as previously defined, may possess a biological activity substantially similar to that of the M171I mutated IFN α -2, i.e. which is lower than that of the natural wild-type IFN α -2.

Said compound may also have a biological activity which is even lower than that of the M171I mutated IFN α -2.

30 Said compound may be a biochemical compound, such as a polypeptide or a peptide for example, or an organic chemical compound, such as a synthetic peptide-mimetic for example.

The present invention also provides a new compound having a cellular antiproliferative activity on Daudi Burkitt's cell line at least 15 times lower than that of the natural wild-type IFN α -2.

35 The present invention also concerns a new compound having a signal transduction capacity on MCF7 cells at least 10 times lower than that of the natural wild-type IFN α -2.

5 The present invention also concerns the use of a polypeptide of the invention containing the M171I SNP, for the identification of a compound such as defined above.

The present invention also concerns a process for the identification of a compound of the invention, comprising the following steps:

a) Determining the biological activity, such as the cellular antiproliferative activity on

10 Daudi Burkitt's cell line or the signal transduction capacity, for example;

b) Comparing:

i) the activity determined in step a) of the compound to be tested, with

ii) the activity of the polypeptide of amino acid sequence ID SEQ N°2, or of amino acid sequence comprising the amino acids included between positions 24 and 188 of the amino acid sequence SEQ ID NO.2;

15 provided that said amino acid sequences comprise the M171I SNP; and

c) Determining on the basis of the comparison carried out in step b) whether the compound to be tested has a substantially similar or lower activity compared to that of the polypeptide of amino acid sequence SEQ ID NO.2, or of amino acid sequence comprising the amino acids included between positions 24 and 188 of the amino acid sequence SEQ ID NO.2; provided that said amino acid sequences comprise the M171I SNP.

20 Preferably, the compound to be tested may be previously identified from synthetic peptide combinatorial libraries, high-throughput screening, or designed by computer-aided drug design so as to have the same tri-dimensional structure as that of the polypeptide of amino acid sequence SEQ ID NO.2, or of amino acid sequence comprising the amino acids included between position 24 and 188 of the amino acid sequence SEQ ID NO.2; provided that said amino acid sequences comprise the M171I SNP.

25 The methods to identify and design compounds are well known to persons skilled in the art.

30 Publications referring to these methods may be, for example:

- Silverman R.B. (1992). "Organic Chemistry of Drug Design and Drug Action". Academic Press, 1st edition (January 15, 1992).
- Anderson S and Chiplin J. (2002). "Structural genomics; shaping the future of drug design? Drug Discov. Today. 7(2):105-107.

- 5 - Selick HE, Beresford AP, Tarbit MH. (2002). "The emerging importance of predictive
ADME simulation in drug discovery". Drug Discov. Today. 7(2):109-116.
- Burbidge R, Trotter M, Buxton B, Holden S. (2001). "Drug design by machine learning:
support vector machines for pharmaceutical data analysis". Comput. Chem. 26(1): 5-14.
- Kauvar L.M. (1996). "Peptide mimetic drugs: a comment on progress and prospects"
10 14(6): 709.

The compounds of the invention may be used for the preparation of a therapeutic composition intended for the prevention or the treatment of one of the diseases selected from the group consisting of disorders and/or human diseases, such as cancers and tumors, infectious diseases, venereal diseases, immunologically related diseases and/or autoimmune diseases and
15 disorders, cardiovascular diseases, metabolic diseases, central nervous system diseases, and disorders connected with chemotherapy treatments.

Said cancers and tumors may include carcinomas comprising metastasizing renal carcinomas, melanomas, lymphomas comprising follicular lymphomas and cutaneous T cell lymphoma, leukemias comprising hairy-cell leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia, cancers of the liver, neck, head and kidneys, multiple myelomas, carcinoid tumors and tumors that appear following an immune deficiency comprising Kaposi's sarcoma in the case of AIDS.

Said infectious diseases include viral infections comprising chronic hepatitis B and C and HIV/AIDS, infectious pneumonias, and venereal diseases, such as genital warts.

Said immunologically and auto-immunologically related diseases may include the rejection of tissue or organ grafts, allergies, asthma, psoriasis, rheumatoid arthritis, multiple sclerosis, Crohn's disease and ulcerative colitis.

Said metabolic diseases may include such non-immune associated diseases as obesity.

Said diseases of the central nervous system may include Alzheimer's disease, Parkinson's
30 disease, schizophrenia and depression.

Said diseases and disorders may also include healing of wounds, anemia in dialyzed patient, and osteoporosis.

5 *Experimental Section.*

Example 1: Modeling of a protein encoded by a polynucleotide of nucleotide sequence containing the g1023a SNP and of the protein encoded by the nucleotide sequence of the reference wild-type gene.

In a first step the three-dimensional structure of IFN α -2 was constructed, starting from
10 that of human IFN α -2 whose structure is available in the PDB database (code 1ITF), using the software Modeler (MSI, San Diego, CA). The mature polypeptide fragment was then modified in such a fashion as to reproduce the mutation M148I. A thousand molecular minimization steps were conducted on this mutated fragment by using the programs AMBER and DISCOVER (MSI: Molecular Simulations Inc.). Two molecular dynamic calculation runs were then carried
15 out with the same program and the same force fields. In each case, 50,000 steps were calculated at 300°K, terminated by 300 equilibration steps.

The result of this modeling is visualized on Figures 1 and 2.

Example 2: Prediction of the targeting of the protein encoded by a polynucleotide of nucleotide sequence containing the c527a SNP and of the protein encoded by the nucleotide sequence of the reference wild-type gene.

The c527a polymorphism involves an A6D polymorphism at the level of the immature IFN α -2 protein. This position is located on the signal peptide comprising the first 23 amino acids of the immature protein. The signal peptide controls the addressing of the protein and the intracellular or extracellular compartment where the mature protein will be expressed.
25

PSORT is a software available in the public domain that analyzes the amino acid sequence of the immature protein and predicts in which compartment the mature protein will be localized (Nakai K and Kanehisa M (1992). A knowledge base for predicting protein localization sites in eukaryotic cells. Genomics 14, 897-911).

30 PSORT predicts that the A6D polymorphism changes the IFN α -2 localization at the cellular level. Indeed, it predicts that the wild-type mature IFN α -2 protein is secreted, meaning an extracellular localization, whereas the D6 mutated IFN α -2 will be in the nuclear and/or the mitochondrial compartments.

5 Even if the A6D mutant of IFN α -2 is probably not affected in its structure and function in comparison to the wild-type protein, its activity will not be found where it normally would have been.

10 Example 3. Prediction of changes in IFN α -2 expression caused by the deletion 139-144del(acttta) in the polynucleotide sequence SEQ ID NO.1.

The effects of the 139-144del(acttta) deletion on putative binding sites have been evaluated after a run in Match (Transfac). Although this deletion is not located in the regulatory region common to all interferons, it cancels three putative binding sites for transcription factors:

15 - the putative binding site for Msx-1 encompasses nucleotides 134 to 142 on the wild-type reference sequence SEQ ID NO. 1. The effect of this deletion on this binding site cannot be estimated properly since this transcription factor is not well known and its function has been proposed to be related to limb development but has not been clearly identified (Hwang et al. (1998). Am. J. Med. Genet. 75:419-423).

20 - the putative binding site for Oct-1 encompasses nucleotides 141 to 163 on the wild-type reference sequence SEQ ID NO. 1. Although this binding site is located at about 400 nucleotides upstream of the transcription initiation site, its deletion may have an important effect since the transcription factor that recognizes this site is involved in different mechanisms of transcription initiation in lymphocytes T and B (Ullman et al. (1991). Science. 254:558-562 ; Kemler et al. (1989). EMBO J. 8:2001-2008 ; Kamps et al. (1990). Mol Cell Biol. 10:5464-5472).

25 - the putative binding site for Hoxa3 encompasses nucleotides 143 to 151 on the wild-type reference sequence ID SEQ N°1. Hoxa3 is a transcription factor that is involved in the regulation of fetal thymic epithelial cells to promote thymocyte development (Su et al. (2001). Dev. Biol. 236:316-329).

30 As a result, the 139-144del(acttta) SNP may affect the normal regulation of IFN α -2 expression.

Example 4: Genotyping of the SNPs t110c, t338a, t363c, c427t, c527a, and g1023a in a population of individuals.

35 The genotyping of SNPs is based on the principle of the minisequencing wherein the

5 product is detected by reading polarized fluorescence. The technique consists of a fluorescent minisequencing (FP-TDI Technology or Fluorescence Polarization Template-direct Dye-terminator Incorporation).

10 The minisequencing is performed on a product amplified by PCR from genomic DNA of each individual of the population. The PCR product is chosen in such a manner that it covers the genic region containing the SNP to be genotyped. After elimination of the PCR primers that have not been used and the dNTPs that have not been incorporated, the minisequencing is carried out.

15 The minisequencing consists of lengthening an oligonucleotide primer, placed just upstream of the site of the SNP, by using a polymerase enzyme and fluorolabeled dideoxynucleotides. The product resulting from this lengthening process is directly analyzed by reading polarized fluorescence.

All these steps, as well as the reading, are carried out in the same PCR plate.

Thus, the genotyping requires 5 steps:

- 1) Amplification by PCR
- 2) Purification of the PCR product by enzymatic digestion
- 3) Elongation of the oligonucleotide primer
- 4) Reading polarized fluorescence
- 5) Interpretation of the reading

20 The genotyping steps 1 and 2 are carried out in the same conditions for each of the SNPs t110c, t338a, t363c and c427t on the one hand and each of the SNPs c527a, and g1023a on the other hand.

25 The steps 3, 4 and 5 are specific to each one of these polymorphisms.

1) The PCR amplification of the nucleotide sequence of the IFN α -2 gene is carried out starting from genomic DNA from 268 individuals of ethnically diverse origins.

These genomic DNAs were provided by the Coriell Institute in the United States.

30 The 268 individuals are distributed as follows:

Phylogenetic Population	Specific Ethnic Population	Total	%
African American	African American	50	100.0
	Subtotal	50	18.7
Amerind	South American Andes	10	66.7
	South West American Indians	5	33.3
Caribbean	Subtotal	15	5.6
	Caribbean	10	100.0
European Caucasoid	Subtotal	10	3.7
	North American Caucasian	79	79.8
Mexican	Iberian	10	10.1
	Italian	10	10.1
	Subtotal	99	36.9
Northeast Asian	Mexican	10	100.0
	Subtotal	10	3.7
Non-European Caucasoid	Chinese	10	50.0
	Japanese	10	50.0
Southeast Asian	Subtotal	20	7.5
	Greek	8	21.6
South American	Indo-Pakistani	9	24.3
	Middle-Eastern	20	54.1
	Subtotal	37	13.8
Pacific Islander	Pacific Islander	7	41.2
	South Asian	10	58.8
Subtotal	Subtotal	17	6.3
	South American	10	100.0
Total	Subtotal	10	3.7
	Total	268	100

5

The genomic DNA coming from each one of these individuals constitutes a sample.

For SNPs t110c, t338a, t363c and c427t, the PCR amplification is carried out starting from the following primers:

10 SEQ ID NO. 7 : Sense primer: GCCTCTTATGTACCCACAAA

SEQ ID NO. 8 : Antisense primer: CACCAGTAAAGCAAAGGTCA

These nucleotide sequences permit amplification of a fragment of a length of 535

5 nucleotides, nucleotide 3 to the nucleotide 537 in the nucleotide sequence SEQ ID NO. 1.

For SNPs c527a, and g1023a, the PCR amplification is carried out starting from the following primers:

SEQ ID NO. 9 : Sense primer: CACCCATTCAACCAGTCTA

SEQ ID NO. 10 : Antisense primer: AGCTGGCATACGAATCAAT

10 These nucleotide sequences permit amplification of a fragment of a length of 655 nucleotides, nucleotide 470 to nucleotide 1124 in the nucleotide sequence SEQ ID NO. 1.

For each SNP, the PCR product will serve as a template for the minisequencing

The total reaction volume of the PCR reaction is 5 µl per sample.

This reaction volume is composed of the reagents indicated in the following table:

15

Supplier	Reference	Reactant	Initial Conc.	Vol. per tube (µl)	Final Conc.
Life Technology	Delivered with Taq	Buffer (X)	10	0.5	1
Life Technology	Delivered with Taq	MgSO ₄ (mM)	50	0.2	2
AP Biotech	27-2035-03	dNTPs (mM)	10	0.1	0.2
	On request	Sense Primer (µM)	10	0.1	0.2
	On request	Antisense Primer (µM)	10	0.1	0.2
Life Technology	11304-029	Taq platinum	5U/ µl	0.02	0.1 U/reaction
		H ₂ O	Qsp 5 µl	1.98	
		DNA (sample)	2.5 ng/ µl	2	5 ng/reaction
		Total volume		5 µl	

These reagents are distributed in a black PCR plate having 384 wells provided by ABGene (ref: TF-0384-k). The plate is sealed, centrifuged, then placed in a thermocycler for 384-well plates (Tetrad of MJ Research) and undergoes the following incubation: PCR Cycles: 1 min at 94° C, followed by 36 cycles composed of 3 steps (15 sec. at 94° C, 30 sec. at 56° C, 1 min at 68° C).

20 2) The PCR amplified product is then purified using two enzymes: Shrimp Alkaline Phosphatase (SAP) and exonuclease I (Exo I). The first of these enzymes permits the

5 dephosphorylation of the dNTPs which have not been incorporated during the PCR amplification, whereas the second eliminates the single stranded DNA residues, in particular the primers which have not been used during the PCR.

This digestion is done by addition, in each well of the PCR plate, of a reactional mixture of 5 µl per sample.

10 This reaction mixture is composed of the following reagents:

Supplier	Reference	Reactant	Initial Conc.	Vol. per tube (µl)	Final conc.
AP Biotech	E70092X	SAP	1 U/ µl	0.5	0.5/ reaction
AP Biotech	070073Z	Exo I	10 U/ µl	0.1	1/ reaction
AP Biotech	Supplied with SAP	Buffer SAP (X)	10	0.5	1
		H ₂ O	Qsp 5 µl	3.9	
		PCR product		5 µl	
		Total vol.		10 µl	

Once filled, the plate is sealed, centrifuged, then placed in a thermocycler for 384-well plates (Tetrad of MJ Research) and undergoes the following incubation: Digestion SAP-EXO: 45 min at 37° C, 15 min at 80° C.

15 The elongation or minisequencing step is then carried out on the product of PCR digested by addition of a reaction mixture of 5 µl per prepared sample.

The minisequencing 3) and the reading steps 4) and interpretation of reading 5) are specific to each SNP t110c, t338a, t363c, c427t, c527a, and g1023a.

20 All these steps are described hereinafter outlining the specific conditions used for each one of these polymorphisms.

3) Minisequencing

The sequences of the two minisequencing primers necessary for the genotyping were

5 determined in a way to correspond to the sequence of the nucleotides located upstream of the site of a SNP according to the invention. The PCR product that contains the SNP being a double stranded DNA product, the genotyping can therefore be done either on the sense strand or on the antisense strand. The selected primers are manufactured by Life Technologies Inc.

10 The following table indicates, for each SNP, the sequence of the minisequencing primers that have been tested and the optimal condition retained for the genotyping:

SNP	Primers tested	Optimal condition retained for the genotyping
t110c	SEQ ID NO. 11: Sense primer: taatttaatttttaattgtt SEQ ID NO. 12: Antisense primer: tcctttgccttcattatac	antisense primer + ddATP-R110 + ddGTP-Tamra
t338a	SEQ ID NO. 13: Sense primer: ctgaaaacccatgtaaagag SEQ ID NO. 14: Antisense primer: tcctttgccttcattatac	sense primer + dTTP-R110 + ddATP-Tamra
t363c	SEQ ID NO. 15: Sense primer: aaagaaagcaaaaagagaag SEQ ID NO. 16: Antisense primer: atgcccctgtgttactttct	sense primer + ddTTP-R110 + ddCTP-Tamra
c427t	SEQ ID NO. 17: Sense primer: tccctatttaaggcttaggca SEQ ID NO. 18: Antisense primer: ttctctgaagacccttgcttt	sense primer + ddTTP-R110 + ddCTP-Tamra
c527a	SEQ ID NO. 19: Sense primer: tacaatggccctgacccttg SEQ ID NO. 20: Antisense primer: ccaggagggccaccagtaaa	antisense primer + ddGTP-R110 + ddTTP-Tamra
g1023a	SEQ ID NO. 21: Sense primer: gttgtcagagcagaaatcat SEQ ID NO. 22: Antisense primer: gttgacaaagaaaaagatct	sense primer + ddATP-R110 + ddGTP-Tamra

The minisequencing of the SNPs was first validated over 16 samples, then genotyped over the set of the population of individuals composed of 268 individuals and 10 controls.

15 The elongation or minisequencing step is then carried out as indicated in the following table:

Supplier	Reference	Reactant	Initial conc.	Vol. per tube (µl)	Final conc.
Own preparation		Elongation Buffer ¹ (X)	5	1	1
Life Technologies	On request	Miniseq Primer (µM) A or B	10	0.5	1
AP Biotech	27-2051 (61,71,81)-01	ddNTPs ² (µM) 2 are non labeled	2.5 of each	0.25	0.125 of each
NEN	Nel 472/5 and Nel 492/5	ddNTPs ² (µM) 2 are labeled with Tamra and R110	2.5 of each	0.25	0.125 of each
AP Biotech	E79000Z	Thermo-sequenase	3.2 U/ µl	0.125	0.4 U/ reaction
		H ₂ O	Qsp 5 µl	3.125	
		digested PCR product		10	
		Total volume		15	

¹ The 5X elongation buffer is composed of 250 mM Tris-HCl pH 9, 250 mM KCl, 25 mM NaCl, 10 mM MgCl₂ and 40 % glycerol.

² ddNTPs : For the ddNTPs, a mixture of the 4 bases is carried out according to the polymorphism studied. Only the 2 bases of interest (wild-type nucleotide / mutated nucleotide) composing the SNP are labeled, either in Tamra, or in R110. For example:

³ For SNP c527a, the mixture of ddNTPs is composed of :

- 2.5 µM of ddATP non labeled,
- 2.5 µM of ddCTP non-labeled,
- 2.5 µM of ddTTP (1.875 µM of ddTTP non labeled and 0.625 µM of ddTTP Tamra labeled),
- 2.5 µM of ddGTP (1.875 µM of ddGTP non labeled and 0.625 µM of ddGTP R110 labeled).

For SNP g1023a, the mixture of ddNTPs is composed of :

- 2.5 µM of ddCTP non labeled,
- 2.5 µM of ddTTP non-labeled,
- 2.5 µM of ddGTP (1.875 µM of ddGTP non labeled and 0.625 µM of ddGTP Tamra labeled).

5 labeled),

- 2.5 µM of ddATP (1.875 µM of ddATP non labeled and 0.625 µM of ddATP R110 labeled).

10 Once filled, the plate is sealed, centrifuged, then placed in a thermocycler for 384-well plates (Tetrad of MJ Research) and undergoes the following incubation: Elongation cycles : 1 min. at 93° C, followed by 35 cycles composed of 2 steps (10 sec. at 93° C, 30 sec. at 55° C).

15 After the last step in the thermocycler, the plate is directly placed on a polarized fluorescence reader of type Analyst® HT of L JL Biosystems Inc. The plate is read using Criterion Host® software by using two methods. The first permits reading the Tamra labeled base by using emission and excitation filters specific for this fluorophore (excitation 550-10 nm, emission 580-10 nm) and the second permits reading the R110 labeled base by using the excitation and emission filters specific for this fluorophore (excitation 490-10 nm, emission 520-10 nm). In the two cases, a dichroic double mirror (R110/Tamra) is used, and the other reading parameters are:

20 Z-height: 1.5 mm

Attenuator: out

Integration time: 100,000 µsec.

Raw data units: counts/sec

Switch polarization: by well

25 Plate settling time: 0 msec

PMT setup: Smart Read (+), sensitivity 2

Dynamic polarizer: emission

Static polarizer: S

30 A file result is thus obtained containing the calculated values of mP (milliPolarization) for the Tamra filter and that for the R110 filter. These mP values are calculated starting from intensity values obtained on the parallel plane (//) and on the perpendicular plane (⊥) according to the following formula:

$$MP = 1000(// - g\perp)/(// + g\perp).$$

35 In this calculation, the value \perp is weighted by a factor g. It is a machine parameter that must be determined experimentally beforehand.

5 4) and 5) Interpretation of the reading and determination of the genotypes.

The mP values are reported on a graph using Microsoft Inc. Excel software, and/or Allele Caller® software developed by L JL Biosystems Inc.

On the abscissa is indicated the mP value of the Tamra labeled base, on the ordinate is indicated the mP value of the R110 labeled base. A strong mP value indicates that the base 10 labeled with this fluorophore is incorporated and, conversely, a weak mP value reveals the absence of incorporation of this base.

Up to three homogenous groups of nucleotide sequences having different genotypes are obtained.

15 The use of the Allele Caller® software permits, once the identification of the different groups is carried out, direct extraction of the genotype for each individual in table form.

Results of the minisequencing for the SNPs t110c, t338a, t363c, c427t, c527a, and g1023a.

After the completion of the genotyping process, the determination of the genotypes of the individuals of the population of individuals for the SNPs studied here was carried out using the graphs described above.

For SNP t110c, the genotype is in theory either homozygote TT, or heterozygote TC, or homozygote CC in the tested individuals. In reality, and as shown below, the homozygote genotype CC is not detected in the population of individuals.

For SNP t338a, the genotype is in theory either homozygote TT, or heterozygote TA, or homozygote AA in the tested individuals. In reality, and as shown below, the homozygote genotype AA is not detected in the population of individuals.

For SNP t363c, the genotype is in theory either homozygote TT, or heterozygote TC, or homozygote CC in the tested individuals. In reality, and as shown below, the homozygote genotype CC is not detected in the population of individuals.

30 For SNP c427t, the genotype is in theory either homozygote CC, or heterozygote CT, or homozygote TT in the tested individuals. In reality, and as shown below, the homozygote genotype TT is not detected in the population of individuals.

35 For SNP c527a, the genotype is in theory either homozygote CC, or heterozygote CA, or homozygote AA in the tested individuals. In reality, and as shown below, the homozygote genotype AA is not detected in the population of individuals.

5 For SNP g1023a, the genotype is in theory either homozygote GG, or heterozygote GA, or homozygote AA in the tested individuals. In reality, and as shown below, the homozygote genotype AA is not detected in the population of individuals.

10 The results of the distribution of the determined genotypes in the population of individuals and the calculation of the different allelic frequencies for the 6 SNPs studied are presented in the following tables:

Phylogenetic Population	Total	t110c								
		f	(95% CI)	TT	%	TC	%	CC	%	Total
African American	50	8.0	(2.7, 13.3)	42	84.0	8	16.0			50
Amerind	15			15	100					15
Caribbean	10			10	100					10
European Caucasoid	99	1.0	(0, 2.4)	97	98.0	2	2.0			99
Mexican	10			10	100					10
Non-European Caucasoid	37			37	100					37
Northeast Asian	20			20	100					20
South American	10	5.0	(0, 14.6)	9	90.0	1	10.0			10
Southeast Asian	17			17	100					17
<i>Total</i>	<i>268</i>	<i>2.1</i>	<i>(0.9, 3.3)</i>	<i>257</i>	<i>95.9</i>	<i>11</i>	<i>4.1</i>			<i>268</i>

Phylogenetic Population	Total	t338a								
		f	(95% CI)	TT	%	TA	%	AA	%	Total
African American	50	17.0	(9.4, 24.6)	31	66.0	16	34.0			47
Amerind	15			15	100					15
Caribbean	10	5.0	(0, 14.6)	9	90.0	1	10.0			10
European Caucasoid	99	0.5	(0, 1.5)	98	99.0	1	1.0			99
Mexican	10			10	100					10
Non-European Caucasoid	37	1.4	(0, 4.0)	36	97.3	1	2.7			37
Northeast Asian	20			20	100					20
South American	10			10	100					10
Southeast Asian	17			17	100					17
<i>Total</i>	<i>268</i>	<i>3.6</i>	<i>(2.0, 5.2)</i>	<i>246</i>	<i>92.8</i>	<i>19</i>	<i>7.2</i>			<i>265</i>

Phylogenetic Population	Total	t363c								
		f	(95% CI)	TT	%	TC	%	CC	%	Total
African American	50			48	100					48
Amerind	15			15	100					15
Caribbean	10			10	100					10
European Caucasoid	99	0.5	(0, 1.5)	97	99.0	1	1.0			98
Mexican	10			10	100					10
Non-European Caucasoid	37			36	100					36
Northeast Asian	20			20	100					20
South American	10			10	100					10
Southeast Asian	17			17	100					17
<i>Total</i>	<i>268</i>	<i>0.2</i>	<i>(0, 0.6)</i>	<i>263</i>	<i>99.6</i>	<i>1</i>	<i>0.4</i>			<i>264</i>

Phylogenetic Population	Total	c427t							Total
		f	(95% CI)	CC	%	CT	%	TT	
African American	50			50	100				50
Amerind	15			15	100				15
Caribbean	10			10	100				10
European Caucasoid	99			99	100				99
Mexican	10			10	100				10
Non-European Caucasoid	37			37	100				37
Northeast Asian	20			20	100				20
South American	10	5.0	(0, 14.6)	9	90.0	1	10.0		10
Southeast Asian	17			17	100				17
<i>Total</i>	<i>268</i>	<i>0.2</i>	<i>(0, 0.6)</i>	<i>267</i>	<i>99.6</i>	<i>1</i>	<i>0.4</i>		<i>268</i>

Phylogenetic Population	Total	c527a (A6D)							Total
		f	(95% CI)	CC	%	CA	%	AA	
African American	50			50	100				50
Amerind	15			15	100				15
Caribbean	10	5.0	(0, 14.6)	9	90.0	1	10.0		10
European Caucasoid	99			95	100				95
Mexican	10			10	100				10
Non-European Caucasoid	37			37	100				37
Northeast Asian	20			20	100				20
South American	10			10	100				10
Southeast Asian	17			17	100				17
<i>Total</i>	<i>268</i>	<i>0.2</i>	<i>(0, 0.6)</i>	<i>263</i>	<i>99.6</i>	<i>1</i>	<i>0.4</i>		<i>264</i>

Phylogenetic Population	Total	g1023a (M171I)							Total
		f	(95% CI)	GG	%	GA	%	AA	
African American	50			50	100				50
Amerind	15			14	100				14
Caribbean	10	5.0	(0, 14.6)	9	90.0	1	10.0		10
European Caucasoid	99			97	100				97
Mexican	10			10	100				10
Non-European Caucasoid	37			37	100				37
Northeast Asian	20			20	100				20
South American	10			10	100				10
Southeast Asian	17			17	100				17
<i>Total</i>	<i>268</i>	<i>0.2</i>	<i>(0, 0.6)</i>	<i>264</i>	<i>99.6</i>	<i>1</i>	<i>0.4</i>		<i>265</i>

- 5 In the above tables,
- N represents the number of individuals,
 - % represents the percentage of individuals in the specific sub-population,
 - the allelic frequency represents the percentage of the mutated allele in the specific sub-population,
- 10 - 95 % IC represents the minimal and maximal interval of confidence at 95 %.

It is necessary to specify that, for example for the c527a SNP that has been genotyped in antisense, the allele g read in antisense corresponds to the allele c read in sense, and to the presence of an alanine at position 6 of the amino acid sequence of the immature IFN α -2 protein and therefore that the allele t read in antisense corresponds to the allele a read in sense corresponding to an aspartic acid for this position in the sequence of the corresponding protein.

15 By examining these results by phylogenetic population, and by SNP, it is observed that:

- for SNP t110c, the 11 heterozygote individuals TC come from the phylogenetic populations African-American, European Caucasoid, and South American in the population of individuals.
- for SNP t338a, 19 heterozygote individuals TA are found; they come from the phylogenetic populations African American, Caribbean, European Caucasoid, Non-European Caucasoid in the population of individuals.

Example 5. Expression of natural wild-type IFN α -2 and M148I mutated IFN α -2 in bacteria

20 a) Cloning of the natural wild-type IFN α -2 and mutated (M148I) IFN α -2 in the prokaryotic expression vector pTrc/His-topo.

The nucleotide sequences coding for the natural wild-type and mutated IFN α -2 protein are amplified by PCR.

25 The PCR primers permitting such an amplification are:

30 SEQ ID NO. 23: Sense primer: CACCCATTCAACCAGTCTA

SEQ ID NO. 24: Antisense primer: AGCTGGCATACGAATCAAT

The PCR products are inserted in the prokaryotic expression vector pTrc/His-Topo under the control of the Trc hybrid promoter, inducible by IPTG (Iso-Propyl-Thio-Galactoside), by TOPOTM-cloning (Invitrogen Corp.).

35 This vector permits the heterologous expression of eukaryotic proteins in the bacteria

5 thanks to the presence of a mini-cistronic unit.

The wild-type protein and the mutated protein are produced in the form of fusion proteins carrying an N-terminal extension composed of a 6-histidine tail and the epitope for a specific antibody.

It is possible to cleave this additional region by using the Enterokinase endoprotease.

10 After checking of the nucleotide sequence of the region of the vector coding for the recombinant proteins, the Top 10 strain of *E. coli* (Invitrogen) is transformed with these recombinant expression vectors.

15 b) Heterologous expression in *E. coli* and purification of the natural wild-type IFN α -2 and mutated M148I mutated polyhistidine IFN α -2

20 Two saturated pre-cultures of 100 ml of LBA medium (Luria Bertoni + 100 μ g/mL ampicillin) containing, respectively, a clone coding for wild-type IFN α -2 or that coding for M148I IFN α -2, were carried out for 24 hours at 30°C at an agitation of 200 rotations per minute (rpm). After 24 hours of growth, the cultures were used to inoculate, at 1/10, 900 mL of LBA medium (preincubated over-night at 37°C).

When the second set of cultures reaches a cellular density corresponding to an optical density of 0.8 measured at a wavelength of 600 nm, the expression of the poly-hystidine proteins is then induced by the addition of IPTG at a final concentration of 1 mM and the culture is kept for 5 hours at 30 °C with an agitation at 200 rpm.

25 The pellet of bacteria obtained after centrifugation at 4000 x g, 30 min, 4 °C, is resuspended in 25 mL of buffer A (Tris 50 mM, pH 8, NaCl 50 mM, imidazole 10 mM, PMSF 0.1 mM pH 8).

30 A preincubation of 30 min in ice in the presence of 0.5 mg/mL of lysozyme and 20 units of DNase I precedes a sonication carried out in three steps with control of the temperature of the sample (a step delivered 240 Watt per impulse of 10 sec with 10 sec stop, for 1 min). The cell suspension is then clarified by centrifugation at 15,000 x g for 30 min at 4 °C.

The centrifugation supernatant is next filtered on 0.22 micrometer-filter.

35 The polyhistidine proteins present are then purified by HPLC on HiTrap™ Nickel Affinity resin (Amersham Pharmacia Biotech) previously equilibrated in 50 mM Tris, 300 mM NaCl pH 8.0 (Buffer B). After copiously washing the column with 1M NaCl in 50 mM Tris pH

5 8.0, the elution of the proteins was induced by a linear gradient of imidazole with concentrations ranging from 0.01 to 0.25 M in buffer B.

The presence of the polyhistidine protein in the collected fractions is verified, on the one hand by SDS PAGE electrophoresis and on the other hand by immunodetection with the aid of a specific antibody directed against the N-terminal end of the fusion protein.

10 At this stage, the protein of interest is pure up to 80%.

The last step of the purification consists of a separation of the proteins on an ion-exchange chromatography column.

15 The fractions containing the fusion protein are injected on an anion-exchange column (MiniQ PE 4.6/50, Pharmacia) that was previously equilibrated in buffer 50 mM Tris pH 8. The elution of the proteins is carried out by the passage of an NaCl gradient ranging from 0 to 500 mM, in buffer 50 mM Tris pH 8.

The purity of the protein of interest is estimated on the SDS/PAGE gel and the protein concentrations were measured by BCA assay (bicinchoninic acid and copper sulfate, Sigma).

20 The purified wild-type and mutated IFN α -2 proteins containing the N-terminal polyhistidine end are used in the test on Daudi cells consisting of measurement of the antiproliferative activity of these two forms of IFN α -2 on the cell growth of the Daudi cell line.

Example 6. Expression of natural wild-type IFN α -2 and M148I mutated IFN α -2 in yeast

25 a) Cloning of the natural wild-type IFN α -2 and M148I mutated IFN α -2 in the eukaryote expression vector pPicZ α -topo

The nucleotide sequences coding for the mature part of the natural wild-type IFN α -2 and M148I mutated IFN α -2 are amplified by PCR.

The PCR primers permitting such an amplification are:

SEQ ID NO. 25: Sense primer: TGTGATCTGCCTCAAACCCACAG

30 SEQ ID NO. 26: Antisense primer: TCATTCCCTACTTCTAAACTTCTTGC

The PCR products are inserted in the eukaryote expression vector pPicZ α -TOPO under the control of the hybrid promoter AOX1 inducible by methanol (TOPOTM-cloning; Invitrogen Corp.).

35 This vector permits the heterologous expression of eukaryote proteins in the yeast *Pichia pastoris*.

5 After checking of the nucleotide sequence of the region coding for the recombinant proteins, the vector is linearized by the Pme1 restriction enzyme, and the *P. pastoris* yeast strain (Invitrogen) is transformed with these recombinant expression vectors.

10 b) Heterologous expression in *P. pastoris* and purification of the natural wild-type IFN α -2 and M148I mutated IFN α -2 proteins.

15 Two saturated pre-cultures of 50 ml of BMGY medium (2% Peptone, 1% yeast extract, 1.34% YNB, 1% Glycerol, 100 mM potassium phosphate, 0.4 mg/Liter biotin pH 6.0) containing a clone coding for natural wild-type IFN α -2 or that coding for M148I mutated IFN α -2, were carried out for 24-48 hours at 30°C at an agitation of 200 rotations per minute (rpm).

20 15 When the culture reaches a saturating cellular density (corresponding to an optical density of 12 measured at a wavelength of 600 nm), it is used to inoculate, at 5 OD/ml, 250 ml of BMMY medium (2% Peptone, 1% yeast extract, 1.34% YNB, 0.5% Methanol, 100 mM potassium phosphate, 0.4 mg/Liter biotin pH 6.0).

25 20 The expression of the protein is then induced by methanol at a final concentration of 1%, for 24 hours at 30 °C, with an agitation of the culture flask at 180 rpm.

Due to the presence of the signal peptide sequence of the "alpha factor" upstream of the coding sequence, the proteins are secreted by the yeast in the culture medium. The alpha factor is naturally cleaved during processing.

25 25 The suspension is centrifuged and the protein is purified by HPLC starting from the obtained supernatant.

In a pre-started step, an ultrafiltration (Labscale, cut-off 5000Da, Millipore) followed by a dialysis permits a ten times concentration of the yeast supernatant in a buffer of 50 mM Tris-Cl pH 9.0, NaCl 25 mM.

30 30 The first chromatographic step permits protein recovery by affinity on a blue sepharose column (Amersham Pharmacia). The presence of the protein in the collected fractions is verified, on the one hand by electrophoresis of SDS PAGE type and on the other hand by immuno-detection by a specific antibody directed against the IFN α -2 protein. At this step, the purity of the protein of interest is higher than 75%.

35 In a second purification step, a gel filtration permits buffer exchange of the collected fractions corresponding to IFN α -2 proteins against 50 mM Tris pH 9.0, NaCl 25 mM.

5 The last step of the purification consists of a separation of the proteins on an ion exchange chromatography column.

10 The fractions containing the recombinant protein are injected on an anion exchange column (ResourceQ 6.0 mL, Pharmacia) equilibrated beforehand in Tris 50 mM pH 9, NaCl 25 mM buffer. The elution of the proteins is carried out by the migration of a gradient between 0,025 and 1 M NaCl in the Tris 50 mM pH 9 buffer.

15 The purity of the protein of interest is estimated on SDS/PAGE gel and the protein concentrations were measured by densitometry (Quantity one, Biorad) and BCA assay (bicinchoninic acid and copper sulfate, Sigma).

20 Purified natural wild-type IFN α -2 and M148I mutated IFN α -2 proteins obtained according to the protocols described in examples 5 and 6, eventually scaled-up to produce higher amount of proteins, are used for the functional tests described below.

25 Example 7. Evaluation of the capacity of natural wild-type and M148I mutated IFN α -2 to activate signal transduction in the breast carcinoma cell line MCF-7.

30 The interferons are known to act through signaling pathways involving the JAK (Janus Kinase) and the STAT (Signal Transducers and Activators of Transcription) proteins. The binding of interferon to its receptor induces phosphorylation of the JAK proteins which in turn activate by phosphorylation the STAT proteins. Activated STAT proteins translocate to the nucleus where they bind to interferon response elements on gene promoters, which stimulates transcription of the respective genes. To study the signaling pathways initiated by interferon, the reporter gene technique was used. The procedure is described below.

35 The breast carcinoma cells MCF-7 (ECACC) were seeded at a density of 1.10^4 cells/well in 96-well plates in RPMI supplemented with 10% fetal calf serum for 24 hours. Cells were then transfected for 6 hours with a reporter gene construct (pISRE-Luc) coding for the Firefly Luciferase placed under the control of the Interferon-Stimulated Response Element (Clontech) using Superfect (Qiagen) according to the manufacturer's instructions. Then, culture media were changed and cells were incubated over-night in a CO₂ incubator at 37°C after which they were stimulated with various doses of wild-type or mutated IFN α -2 protein for 6 hours at 37°C. After stimulation, culture media were discarded and replaced with 100 μ l/well of Phosphate Buffered

5 Saline (PBS)/1mM MgCl₂. Luciferase activity was measured in a MicroBeta counter (Perkin-Elmer) following addition of 100µl/well of the substrate Luclite-Plus (Packard).

Results are expressed as the percentage of maximal stimulation of the Luciferase activity. The measurements were performed three times, and the values given here represent the means of the triplicate determinations.

10 The ability of the wild-type IFNα-2 or M148I mutated IFNα-2 to trigger the signal transduction cascades is based on the measurements of their Efficacy Doses at 50% (EC50's) corresponding to their respective concentrations stimulating 50% of the Luciferase activity (the maximal stimulation is considered as being 100% activity).

The average EC50 value measured for the wild-type IFNα-2 is 12.33 pM.

15 The average EC50 value measured for the M148I mutated IFNα-2 is 122 pM.

Thus, the ratio corresponding to the EC50 value for the mutated protein over the EC50 value for the wild-type protein reaches 9.91 (with a standard deviation of 3.4).

Consequently, this test demonstrates that the biological activity of M148I mutated IFNα-2 is 10 times less than that of wild-type IFNα-2 based on its capacity to activate the interferon signaling pathway.

Example 8. Evaluation of immunomodulatory activity of natural wild-type IFNα-2 and M148I mutated IFNα-2

IFNs type I (IFN alpha and IFN beta) are able to modulate certain functions of the immune system. They have been demonstrated to increase the dendritic cells (DC) maturation: increase in the expression of MHC class I (HLA ABC) and II (HLA ADR) molecules, increase in the expression of the molecules involved in the co-stimulation of the T-lymphocytes, CD80, CD86 and CD83 molecules and increase in the stimulating function of T-lymphocyte.

30 a) Effect of natural wild-type IFNα-2 and M148I mutated IFNα-2 on dendritic cell maturation.

Immunomodulatory activity of wild-type IFNα-2 and M148I mutated IFNα-2 was first investigated on dendritic cells maturation. To do so, dendritic cells were first generated from adult peripheral blood monocytes cultivated in the presence of GM-CSF and IL-4 cytokines.

5 After purification using a CD14+ cells purification kit, these dendritic cells were placed in presence of 100 ng/mL of wild-type IFN α -2 or M148I mutated IFN α -2 and their phenotype was determined by FACS analysis aiming at looking for the expression of the MHC class I and II molecules and the CD40, CD80, CD86, CD83, and CD1a markers. The maturation state of these dendritic cells has also been compared to that obtained without IFN α -2 treatment, to provide a
10 control with non-stimulated dendritic cells.

The median value of the measures of fluorescence intensity for each marker and for the three experimental conditions, expressed as arbitrary unit, are presented in the following table:

	HLA ABC	HLA ADR	CD40	CD80	CD86	CD83	CD1a
No Stimulation	64	133	24	25	14	15	26
Wild-type IFN α -2	87	281	331	76	45	15	155
M148I IFN α -2	86	155	149	40	15	16	202

b) Effect of natural wild-type IFN α -2 and M148I mutated IFN α -2 on cytokine release by T lymphocytes.
15

Immunomodulatory activity of wild-type IFN α -2 and M148I mutated IFN α -2 was also investigated by measuring cytokine release by T lymphocytes placed in presence of wild-type IFN α -2 and M148I mutated IFN α -2 and with or without a strong antigen (SEB) in order to mimic an immune response against an aggression.

To do so, peripheral blood mononuclear cells (PBMC) were isolated from healthy donors and stimulated for 16 hours in an appropriate medium containing anti-CD3 and anti-CD28 antibodies or SEB. In each culture was added 4 μ g/mL of wild-type IFN α -2 or M148I mutated IFN α -2. After stimulation, T lymphocytes were extracellularly labeled with anti-CD3, anti-CD4 and anti-CD69 antibodies or anti-CD3, anti-CD8 and anti-CD69 antibodies, and intracellularly labeled with specific antibodies directed against Th1-type cytokines (IFN-gamma) or Th2-type cytokines (IL-10).
20
25

Fluorescent cells were analyzed using FACScalibur and CellQuest software.

The results obtained indicate that both M148I mutated IFN α -2 and wild-type IFN α -2 do not stimulate IL-10 and IFN-gamma release and, thus, do not activate T lymphocytes in absence of SEB. In contrast, both M148I mutated IFN α -2 and wild-type IFN α -2 stimulate cytokines (IL-
30

- 5 10 and IFN-gamma) release by SEB-activated T-lymphocytes as shown in the table below. This table represents the cytokine release by T-lymphocytes in presence of SEB, expressed as percentage of the CD4+ CD69+ cells or CD8+ CD69+ cells for the CD4+ T-lymphocytes and CD8+ T-lymphocytes, respectively, and the percentage of CD69+ cells on total cells.

T-lymphocyte		IFN gamma	IL-10	CD69+ cells/total
CD4+ CD69+	Negative control	11.9	7.5	1.26
	Wild-type IFN α -2	19.6	24.68	2.7
	M148I mutated IFN α -2	24.03	10.3	2.6
CD8+ CD69+	Negative control	8.73	0.65	4.69
	Wild-type IFN α -2	16.37	4.26	10.02
	M148I mutated IFN α -2	24.84	3.4	10.51

c) Effect of natural wild-type IFN α -2 and M148I mutated IFN α -2 on cytokine release by monocytes.

Finally, immunomodulatory activity of wild-type IFN α -2 and M148I mutated IFN α -2 was investigated by measuring cytokine release by monocytes in absence or in presence of a bacterial toxic agent (LPS).

To do so, human peripheral blood mononuclear cells (PBMC) were isolated from healthy donors and their phenotype was analyzed to determine the relative amount of CD64+ CD4dim cells (CD64 and CD4dim are markers for blood monocytes). After an over-night culture, these PBMC were incubated in the culture medium alone (not stimulated cells) or in presence of LPS (stimulated cells). In each culture, 4 μ g/ml of wild-type IFN α -2 or M148I mutated IFN α -2 was added. After culture, cells were extracellularly labelled with anti-CD64 and anti-CD4dim, and intracellularly labelled with specific antibodies directed against Th1-type cytokines (TNF-alpha), IL-12 and IL-10.

Fluorescent cells were analyzed using FACScalibur and CellQuest software.

The results obtained indicate that both M148I mutated IFN α -2 and wild-type IFN α -2 do

5 not stimulate cytokines (IL-10, IL-12 and TNF-alpha) release in absence of LPS. In contrast, in presence of LPS, both M148I mutated IFN α -2 and wild-type IFN α -2 stimulate cytokines (IL-10, IL-12 and TNF-alpha) release by monocytes as shown in the table below. This table represents cytokine release by monocytes in presence of LPS, expressed as percentage of the CD64+ CD4dim cells, and the percentage of CD4dim CD64+ cells on total cells.

10

	IL-10	IL-12	TNF- α	CD4dim CD64+ cells/total
No stimulation	16.21	8.52	13.88	3.1
Wild-type IFN α -2	49.34	34.48	50.87	2.71
M148I mutated IFN α -2	42.4	27.86	60.14	2.85

Example 9. Evaluation of *in vitro* antiproliferative activity of natural wild-type IFN α -2 and M148I mutated IFN α -2.

a) Antiproliferative activity of natural wild-type IFN α -2 and M148I mutated IFN α -2 on the human lymphoblasts of Daudi Burkitt's cell line proliferation

These tests are carried out on two different types of IFN α -2, namely: natural wild-type IFN α -2 and M148I IFN α -2. Cells (human Daudi Burkitt's lymphoma cell line, hereinafter called "Daudi cells") cultivated beforehand in a RPMI 1640 medium (supplemented with 10% fetal calf serum and 2 mM of L-Glutamine) are inoculated in 96-well plates at the cellular density of 4.10^4 cells/ well.

In each well, Daudi cells are placed in contact of increasing concentrations of either natural wild-type or mutated IFN α -2.

For each of the wild-type and mutated IFN α -2, final concentrations of 0.003 pM to 600 nM are tested.

Eight different cultures, and therefore different measurements, are carried out in parallel for both proteins and for each concentration.

The Daudi cells are then incubated for 66 h at 37 °C under 5% CO₂ after which the Uptiblue reagent (Uptima) is added to the cultures. The rate of cell proliferation is quantified by measuring the fluorescence emitted at 590nm (excitation 560nm) after an additional period of incubation of 4

5 hours.

The antiproliferative activity of the wild-type IFN α -2 or M148I mutated IFN α -2 is based on the measurements of the IC₅₀ corresponding to the concentration of IFN α -2 inhibiting 50% of the cell growth.

The results obtained are illustrated in Figure 3.

10 For each concentration of wild-type and mutated (M148I) proteins, the points represented on the graph are the mean of four measurements performed on the four cultures made in parallel for each of the proteins and concentrations.

15 The average IC₅₀ value measured for the wild-type IFN α -2 is 0.3, whereas the average IC₅₀ value measured for the mutated M148I IFN α -2 is 5.0. Thus, the ratio corresponding to the value of the IC₅₀ of the mutated protein over the value of the natural wild-type protein reaches 15.3.

This test demonstrates that the cellular antiproliferative activity is greatly decreased in the case of M148I mutated IFN α -2 by comparison with wild-type IFN α -2.

20 b) Antiproliferative activity of natural wild-type IFN α -2 and M148I mutated IFN α -2 on the TF-1 erythroleukemia cell line.

25 The effect of the wild-type and mutant IFN α -2 was also evaluated on TF-1 erythroleukemia cell line. To do so, TF-1 cells were placed in contact of increasing concentrations of either natural wild-type or mutated IFN α -2 (0.001 to 1000 ng/mL) and the cell proliferation measured.

30 The results are expressed as the IC₃₀ corresponding to the IFN α -2 concentration inhibiting proliferation of 30% of cells. The IC₃₀ measured for the wild-type IFN α -2 is 0.66, and the IC₃₀ measured for the mutant IFN α -2 is 3.09. These data indicate that the M148I mutated IFN α -2 has a weak antiproliferative effect on TF-1 cells, and this effect is similar to that of wild-type IFN α -2, suggesting that the M148I mutated IFN α -2 hematologic toxicity is not superior than that of wild-type IFN α -2.

Example 10. Evaluation of the antiviral activity of natural wild-type IFN α -2 and M148I mutated IFN α -2.

5 The IFNs play an important role in antiviral defense. IFN antiviral activity is partly due to IFN-induced enzymatic systems, such as:

- The 2'5' oligoadenylylate synthetase, an enzyme which catalyzes the adenosine oligomeric synthesis. These oligomers activate the RNase L, an endoribonuclease which destroys the viral RNA once activated.

10 - The Mx proteins (GTPases) which inhibit the synthesis and/or the maturation of viral transcripts. This activity is mainly exerted on the influenza virus.

- The PKR protein (or p68 kinase) which is activated by the double-stranded RNA. The activated PKR inhibits proteic synthesis.

15 The IFN's antiviral activity is also induced by other mechanisms such as, in the case of retroviruses, the inhibition of viral particles entry into the cells, the replication, the binding, the exit of the particles and the infective power of viral particles.

20 Finally, the IFNs exert an indirect antiviral activity by modulating certain functions of the immune system, in particular by favoring the response to cellular mediation (including increase of the MHC class I and II molecules, increase of IL-12 and IFN-gamma production, increase of the CTL activities, among others).

The antiviral activity of M148I mutated IFN α -2 has been evaluated both *in vitro* in cell culture and *in vivo* in mouse model.

a) Antiviral activity *in vitro* in cell culture.

25 This assay permits evaluation of the antiviral activity of natural wild-type IFN α -2 and M148I mutated IFN α -2 in cell culture using the vesicular stomatitis virus (VSV).

To do so, WISH human epithelial cells are cultivated for 24 hours in the presence of decreasing concentrations of M148I mutated IFN α -2 and wild-type IFN α -2. Then, the cells are infected by the virus of vesicular stomatitis (VSV) during 24 to 48 additional hours and the cell lysis is measured.

30 The antiviral effect of the different IFN α tested is determined by comparing the IC50 value corresponding to the IFN concentration inhibiting 50% of cell lysis induced by the VSV.

A similar experiment has been carried out two times, and the average results indicate that the IC50 reaches 4 ng/ml in the case of wild-type IFN α -2 and 22 ng/ml in the case of M148I mutated IFN α -2. Thus, in cell culture infected with VSV, the M148I mutated IFN α -2 has a lower antiviral activity than the wild-type IFN α -2.

5

b) Antiviral activity *in vivo* in mouse model.

This test *in vivo* is performed in EMCV (Encephalomyocarditis virus) mouse model.

Human IFNs nevertheless exhibit dose-dependent antiviral activity in the mouse which is in general 100 to 1,000 fold less than that exhibited by the same amount of mouse IFN (Meister et al. (1986). J. Gen. Virol. 67, 1633-1644).

Intraperitoneal injection of mice with Encephalomyocarditis virus (EMCV) gives rise to a rapidly progressive fatal disease characterized by central nervous system involvement and encephalitis (Finter NB (1973). Front Biol. 2: 295-360). Mouse and human interferon-alpha have both been shown to be effective in protecting mice against lethal EMCV infection (Tovey and Maury (1999). J. IFN Cytokine Res. 19: 145-155).

Groups of 20, six-week old Swiss mice were injected intraperitoneally (ip) with 100 x LD₅₀ EMCV and treated one hour later, and then once daily for 3 days thereafter with 2 µg of M148I mutated IFN α -2 and wild-type IFN α -2 preparations. A control group was performed with animals having been treated with excipient only. The animals were followed daily for survival for 21 days.

Results are presented in Figure 4 and indicate that the relative survival rate of the mice which have been treated with M148I mutated IFN α -2 is much higher than the survival rate of the non-treated mice but remains similar to that observed for the mice which have been treated with wild-type IFN α -2.

Thus, this test demonstrates that the antiviral activity in EMCV mouse model of M148I mutated IFN α -2 is similar to that of the wild-type IFN α -2.

All of these results demonstrate that M148I mutated IFN α -2 possesses unique biological properties.

30

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but merely as an exemplification of the various embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended hereto.